UNIVERSITY OF NEW ZEALAND.

THESIS

Presented in partial fulfilment of

the requirements for the Degree

of Master of Science with

Honours in Zoology.

# STUDIES ON THE BIOLOGY AND FUNCTIONAL MORPHOLOGY OF TRIPLECTIDES OBSOLETA

By

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## INTRODUCTION.

The most significant of recent contributions to the knowledge of the Trichopterous fauna of New Zealand has been that of Mosely and Kimmins (1953), a systematic account of families, genera and species of adult forms, which is of considerable help to workers on the order in New Zealand. A further systematic work which is useful is that of Hickin (1946, 1949), who gives descriptions and keys to the larvae and pupae of the families of British Trichoptera. A key to the spawn is given by Siltala (1906), for determination also to the family level. Lestage (1921) and Lloyd (1921) give systematic accounts of European and North American larval forms respectively. A general description of the family is given, and this is followed by descriptions of the species of different genera characteristic to the two countries. Betten (1934) in his extensive work on the caddisflies of the New York State, gives keys to adults, larvae and pupae of the different families; further keys are given within each family, to the genera and sometimes species. Betten also gives an extensive review of the literature pertaining to the Trichoptera up until that time.

Biological, morphological and ecological studies have mainly been confined to the European and North American forms. Lestage (1921) gives an account of the morphology of the larva and pupa. This is followed by a large section on the biological aspects of spawning; larval life, which includes sections on respiration, feeding, cases and larval shelters; pupae and pupation; and emergence. This work is very general and deals mainly with the larval and pupal stage. Lloyd

(1921) gives a similar account of the North American forms. Betten's (1934) work was originally written in 1906-17, and was revised and brought up to date several times before its final publication in 1934. This work is mainly a systematic one, this part being preceded by sections on the geographical distribution of the Trichoptera and the morphology of the Trichoptera, which includes contributions by Kjellgren on the antennae of the adults and Orcutt on the Larvae, the section as a whole giving the main morphological features of the larva, pupa and adult. A further section preceding the main systematic part is that of Davis on the habits of Trichoptera, which includes sub-sections on the adult, larva and pupa; finally there is a section dealing with the Relationships of the Trichoptera. As a whole this work contributes a great deal to the knowledge of Trichoptera. Despax (1951) also gives a fairly extensive contribution to the literature. He describes the internal anatomy as well as the external anatomy of the adult, and aspects of ecology and ethology. The internal and external anatomy is given also for the larva. Finally the morphology and biology of the pupa is described, small sections on enemies and parasites are given for the larval and adult stages. Nielsen (1942, 1948) is concerned with the development and biology and the phylogeny also of Trichoptera, and he deals also with the interesting question of the origin of the case building instinct. Crichton (1957) gives a detailed account of the structure and function of the mouth parts of Phryganea striata L., followed by a comparative study of these structures throughout the order while observations on the feeding of caddisflies are reviewed. There is little literature accounting for the larval and adult internal anatomy of Trichoptera in any detail. One of the main works is on the New Zealand species Hydropsyche colonica (Glasgow 1936) in which the internal anatomy of the larva is described in some detail, together with a brief account of the internal anatomy of the adult. Deoras (1944) describes the adult internal anatomy of eight species, in his work on the comparative morphology of adult Trichoptera, in which he aimed to give some evidence of their evolution.

Work on the order in New Zealand, with the exception of Glasgow, has been systematic: Mosely and Kimmins (1953), Tillyard (1924, 1926), and McFarlane (1951 and 1939). Some life histories have been briefly described, Hudson (1904) in particular, and McFarlane (1936, 1939 and 1951) is mainly concerned with the systematics of the Rhyacophilidae, but mentions also the outline of life histories of some of the species. No work of any detail on the life history of the New Zealand Leptoceridae or any other aspects of this family other than systematic has to my knowledge been undertaken.

The main locality for obtaining material for work on this thesis was the Styx River. A subsidiary area, a tributary of the Hawdon River, Cass, was also visited during the year. Regular samplihg of all stages of the life history was almost wholly confined to the upper branch of the Styx River, the source of which lies in a drain about 1 mile east of the Harewood airport. The stream runs through dairy farms, fruit farms and market garden land and receives a certain amount of drainage from them. For most of its course it flows over a deep bed of silt and

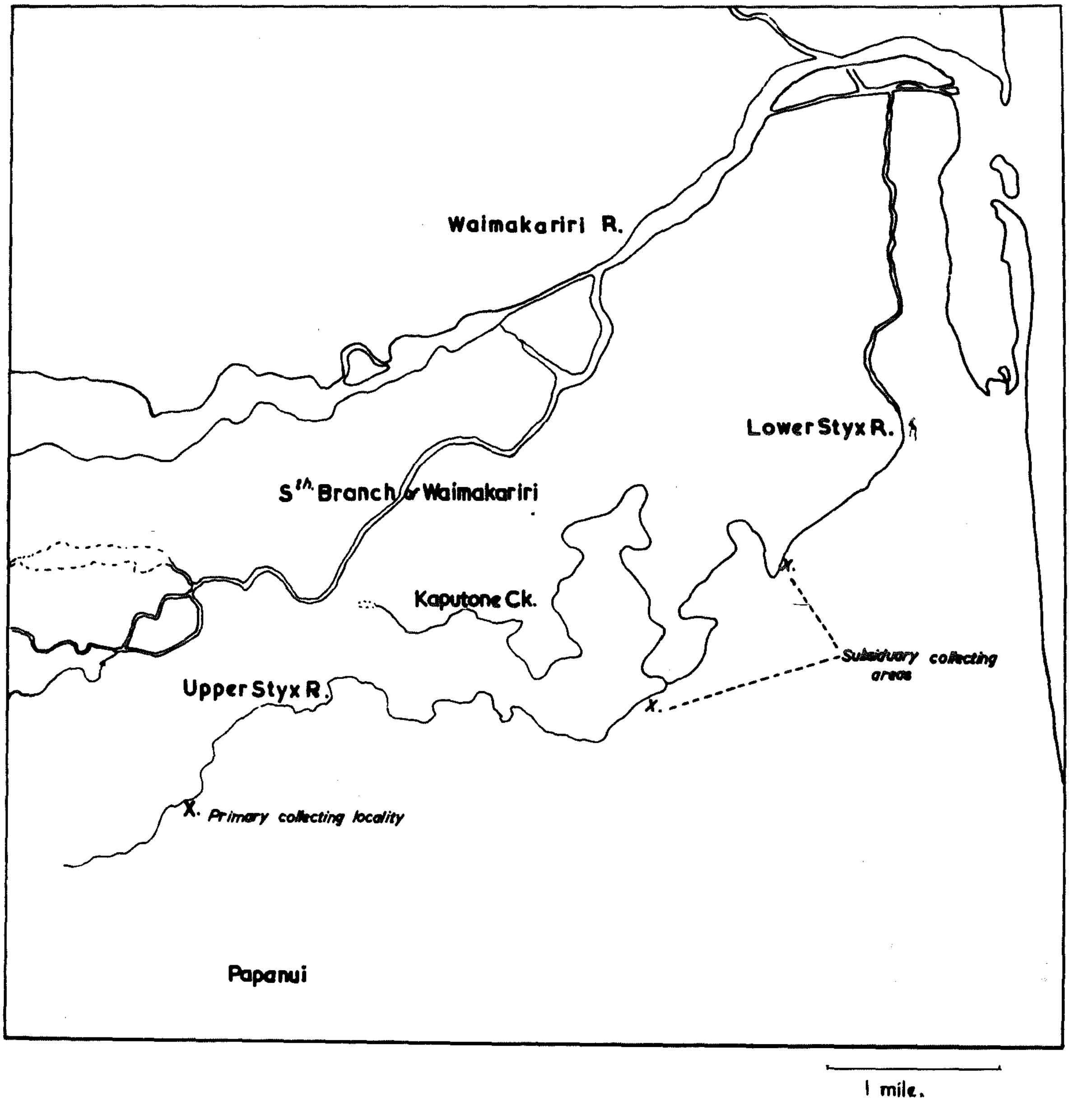


Fig.I.a. Map to show collecting localities.

an occasional band of shingle which normally lies beneath the layer of silt. The river is supplied with water from springs which are found frequently along the upper reaches of the river, where the water is from 1 - 4 ft. deep and from 3 - 9 ft. wide. After meeting the Kaputone Creek tributory, the Styx River becomes a much wider and deeper water course, and flows at a slow rate in a north east to northerly direction out to sea near the mouth of the Waimakariri River (Fig.1 A). The whole course of the river flows over land which does not reach 100 ft. above sea level. The water of the river just below the meeting of the two tributaries is frequently discoloured. No extensive work has been done in this area as it is too deep and inaccessible in most places for a reliable and satisfactory source of material. However larvae were taken during the months of May, June and July, by dragging a net through the Elodea along the edges of the river. These samples provided information concerning certain aspects of the larval case building habits, and material from the Upper Styx was also used for this purpose. The river throughout most of its length is lined with willows (Salix sp.) and in the area of the primary collecting locality Hoheria sexstylosa, Elderberry (Sambicus niger) and Lecesteria sp. are common species. The vegetation provides a fairly dense cover to the stream beneath (Frontpiece) and also contributes a considerable supply of debris to the stream, whereby an ideal habitat is provided for the several stages in the life history of this species.

Material was found to be readily available from the Upper Styx branch of the Styx River approximately one mile from its origin. Larval samples were collected by netting samples from the stream bed, and by hand picking the larvae from sodden logs of wood. Samples and individually picked larvae were taken back to the laboratory in plastic bags, sorted, and fixed and preserved for future use. Larval material was found to travel best in hot weather if placed in a moist plastic bag with only a small amount of water. Live material has been kept all year in well aerated tanks for various observations and experiments.

Pupal material was readily collected from inside submerged or partly submerged logs, of relatively soft texture, while less frequently they were located in the old larval cases. Pupae were kept in running water aquaria, and several emerged quite successfully in the laboratory.

The imagos were found flying on calm nights at dusk, and up to approximately 2 hours afterwards. It seemed that they are only moderately attracted to light, but enough for them to be swept up with a butterfly net, while in flight. This is usually in close proximity to the stream. The light used was that of a 300 candle power paraffin tilley lamp. The adults were caught individually from the folds of the net with an aspirator made out of a jar fitted with a cork through which passed the tubes, and dry grass was placed in the jar so that the adults had some means of support. As soon as about six specimens were caught, a solid cork was fitted to the jar instead of the aspirator apparatus; this was transferred to a second jar ready for further use. The adults were either freed into an insectory, or fixed straight away in Carnoy, and preserved in 70% alcohol.

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The original purpose of this thesis was to study the biology of

Triplectides obsoleta, including the life history with special reference to the reproduction which was supposedly viviparous. As a consequence of this, morphological adaptations of the female imago would have been a very interesting study. However, as investigations proceded it became evident that the female of this species was oviparous, and the viviparous specimens were later identified as Triplectides magna.

The outcome of this has been that other interesting features of the larval, pupal and adult forms as well as an account of the life history has led to a more generalised biological and to a lesser extent morphological study of this insect.

# Chapter 1: LIFE HISTORY.

## 1/1 The Larval Stages

### i. Introduction.

*Triplectides obsoleta* overwinters in the larval state, and larval stages may therefore be found at all times of the year. The first part of this section entails a description of the external features of the larval form. It appeared that the larvae changed little in form throughout their larval life, for no obvious features could be found which differed greatly from one instar to the next, the main change being a gradual increase in size due to normal growth processes.

The primary aim of this section was to determine the number, and if possible the duration of the larval instars; secondly that the instars may be distinguished from each other so that by sampling the population over the autumn, winter and spring months the changes in proportion of the instars represented in the population during this time could be shown. Simultaneously an indication of the larval growth and habitat distribution has been obtained. The larval case and the case building habit is dealt with in a later section.

The egg mass of *T. obsoleta* was not found in the field, but two masses were laid by one female in the laboratory. A description of these and the contained eggs is given at the end of 1/3. The 1st instar larva partly on account of the difficulty in obtaining egg stages has not been found. Consequently its description has had to be omitted from this work.

## ii. Material and Methods

In order to show that samples taken from the stream at monthly intervals should be comparable, an iron frame, 1 foot square was used. This was placed on the stream bed and all the sticks, leaves and other debris lying within the square were scraped into a triangle framed net made out of heavy calico and 1 mm mesh nylon cloth. The net was held by a handle attached to the apex of the triangle, while the base was held firmly opposed to the down stream side of the square, and by this means all the debris carried into the net by the current was collected.

Because the larval case is composed of vegetable material it was almost impossible to find the larvae amongst all the debris without close inspection and careful sorting. Consequently the material was ca rried back to the laboratory in plastic bags and carefully sorted out in flat, white dishes. A small amount of material was placed in a dish with the bottom just covered with water. After a few minutes the larvae were easily discernable walking about the dish. Larvae and cases were placed in 70% alcohol, and were measured immediately, before they had a chance to harden.

The length of the larvae was measured to the nearest millimetre on a white plastic ruler. The length was taken from the anterior margin of the fronto clypeus to the posterior margin of the dorsal anal sclerite of the ninth abdominal segment.

Larval head widths were measuredto .1 mm. by means of a monocular microscope fitted with a micrometer eye piece. Since the head is strongly hypognathous and heavily chitinised it was difficult to hold in position so that an accurate measurement of head width could be made. A shallow petri dish was one-third filled with melted paraffin, when hardened enough water was poured in so as to cover the larvae. By placing fine forceps on either side of the larva in the neck region the head was squeezed forward into the prognathous position, so that the dorsal surface was in full view. The position of the larva was then steadied by applying gentle pressure causing the tips of the forceps to dig into the wax. Head width measurements were made across the widest part of the head: this is level with the area in which the two frontal sutures diverge from the coronal suture.

An investigation of the duration of instars was undertaken by isolating larvae in individual floats. These were made out of cork rings, $\frac{1}{4}$" wide, $\frac{1}{4}$" thick and 2" in diameter. Affixed to the inner surface by means of melted wax was a 1mm mesh nylon cloth net which acted as a "hold". Such a method was described by Grieve (1937) for rearing nymphs of Odonata. Several of these were made and larvae placed in them with some Elodea and a few pieces of wood. A small flag indicating the number of the specimen and the date was pinned on the cork ring. The net hold was at first made out of bolting silk, but this was too fine to allow an adequate circulation of water. Instead the 1mm mesh nylon was used. It was hoped that the cast exuviae would be caught up by the net and thereby an indication of the number and duration of larval instars could be calculated. The floats plus the larvae were placed in running water aquaria and were examined weekly.

Material used for examination of the larval exoskeleton was macerated in a 10% solution of potassium hydroxide in a conical flask. When cleaned the specimens were neutralized in glacial acetic acid, washed in water and changed to 70% alcohol. Parts of the exoskeleton could then be easily separated, and mounted in polyvinyl alcohol type $MA_2$.

Some specimens were stained in a 2% solution of Aniline blue in a lactophenol solution (1 part lactic acid: 1 part phenol) for 2 - 3 mins. (Salmon and Ralph 1955). Excess stain was then washed out in lactophenol solution and specimens were mounted in polyvinyl alcohol $MA_2$. This method proved useful for staining the maxillolabium, increasing denseness of the chitinised parts and staining areas with little chitin, so that their limits were more readily discernable. For the remainder of the exoskeletal examination, however, there was no real advantage in staining the material, as all chitinous parts were readily seen.

It was later realised that material need not be cleaned in potassium hydroxide, as one of the properties of the polyvinyl alcohols is their strong cleaning action, especially in the type used, i.e. $MA_2$ (Salmon 1951). Consequently specimens were mounted straight from 70% alcohol. The larva used for whole mount preparations soon relaxed and extended so that when completely cleaned the different parts were suitably spread for examination. The cleaning process was speeded up as suggested by Salmon if slides were placed in a paraffin embedding oven for approximately 8 hrs.

iii. <u>Description of the larval form.</u>

The larva of <u>T. obsoleta</u> is eruciform, and the head is hypognathous. The pronotum and mesonotum are composed of chitinous sclerites, while the

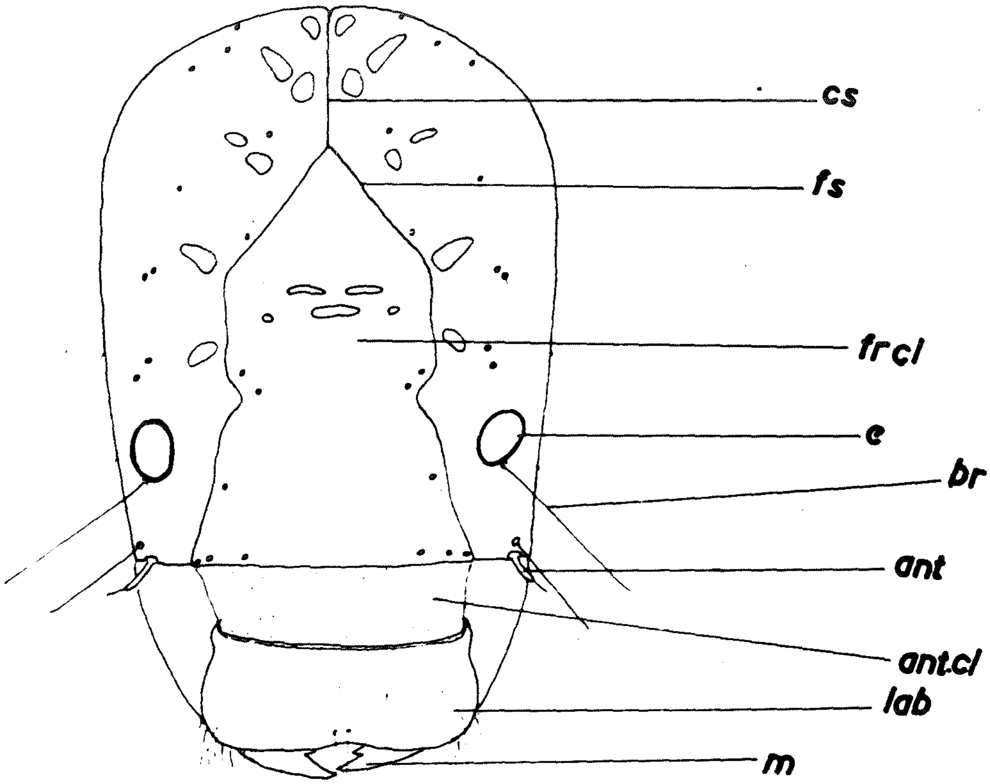



Fig.1. Dorsal view of head.

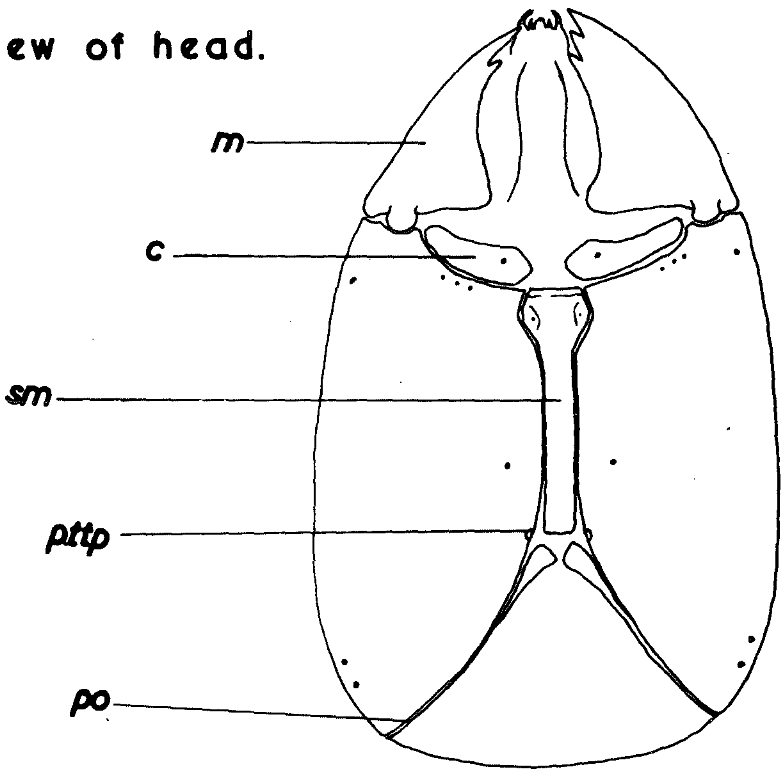



Fig.2. Ventral view of head.

metanotum is membranous except for six small sclerites. The abdomen is a creamy white colour, the segments are clearly demarcated, and the abdomen is extremely contractile. The ninth abdominal segment is narrower than those preceding it, and terminates in a pair of prolegs (Orcutt 1934) each bearing a hook. The fully grown larva measures about 20mm in length and the abdomen 3mm in width. The abdominal length measures up to three times the total length of the head and thorax in the final instar.

The Head.

The head capsule is heavily chitinised, and is dark brown to black in colour, with numerous oval non-pigmented areas (Fig.1). It is cone shaped with the apex flattened. As in all other Trichopterous larvae the head capsule is made up of three distinct sclerites, the homologies of which have caused some discussion.

The frontoclypeus (Fig.1 frcl) which is longer than it is broad has a typical arrangement of 10 bristles. Earlier workers such as Ulmer (1909) and Lestage (1921) refer to this sclerite as 'clypeus'. Orcutt (1934) refers to it as frons, but Hickin (1945) points out that according to Snodgrass the frons has the muscles of the labrum attached, and he states that he has not been able to make out this musculature: he therefore designates the term clypeus to this sclerite. Macdonald (1950) maintains that among other evidence to the contrary Das (1937) has shown in Anabolia that there are some labral muscles among the muscles of this sclerite, and it should therefore be better termed frontoclypeus. Upon dissection of T.obsoleta it was found that both the anterior and posterior labral muscles are present; these arise from the central area of the triangular portion of the fronto-clypeus (Fig.1 fr.cl.) According to Snodgrass then, this posterior portion could be called frons, and as the dorsal muscles of the buccal cavity also have their origin on this sclerite, it would seem only logical for it to be/re ferred to as the fronto-clypeus.

The lateral areas of the head capsule are bounded by the curved epi-cranial sclerites. The epistomal suture is divided into a dorsal coronal suture (Fig.1 c.s.) extending back to the occipital foramen, and the two arms of the frontal suture (Fig.1 f.s.). Ventrally the genae are separated to varying degrees by the submentum (Fig.2 s m.), which is subquadrate, and is incomplete in the early instars. The postocciput is more clearly demarcated in the early instars where it is produced into a wedge shaped area ventrally (Fig.2 p o.).

There is a considerable difference in opinion as to the true homology of the sclerite here called submentum. It is frequently called gula or gular sclerite by Trichopteran writers. Das (1937) maintains that the true gular sclerite is absent in Trichopterous larvae: "The so called gula in larval Trichoptera is really the submentum, which lies between the two hypostomal lobes but varies much in shape and relative position". In T.obsoleta larva the tentorial pits lie at the posterior margin of the head capsule, and the premental muscles also originate in this area, just posterior to the submentum, or 'gula' of other writers. According to Das the gula is always proximal to the tentorial pits, never distal, and the origin of the various premental muscles marks the area of the pre-gula suture so that the sclerite lying distal to it will be the submentum, that lying proximal to it, if it exists, will be the gula. It would seem, therefore, that the true gula is indeed absent in this larva. Das shows that the gula is derived from the neck membrane behind the postmentum or submentum. This agrees with Snodgrass (1935), who considers the origin of the gula as being generally associated with the prognathous condition. The membrane on the ventral side of the head behind the postmentum or submentum becomes sclerotised and forms a single median plate, the gula.

It would seem obvious from results of the above mentioned works on this species, that this sclerite is not truly homologous with the gula of other insects, and although it has been called gula by Hickin (1946), Orcutt (1934) and Nielsen (1942) it seems more likely homologous with the submentum of other insects. As Siltala (1907) in Hickin (1946) points out, the sclerite may not be strictly homologous throughout the order, so that what would appear to be submentum here, may not be so in other species.

The epicranial sclerites have at the most 20 bristles arranged in a definite pattern (Fig.1 br.). The oval light patches correspond mostly to the mandibular muscle attachments and therefore remain fairly constant throughout the larval life. Laterally behind the subgenal suture are the antennae (Fig.1 ant.). They consist of a rounded basal segment and an elongate distal segment carrying a sensory bristle (Hickin 1946). The eyes (Fig.1 e.) are situated slightly median to and behind the antennae, appearing as a group of darkly pigmented spots surrounded by a clear area. According to Wesenberg-Lund (1911 c) in Orcutt (1934) there is some

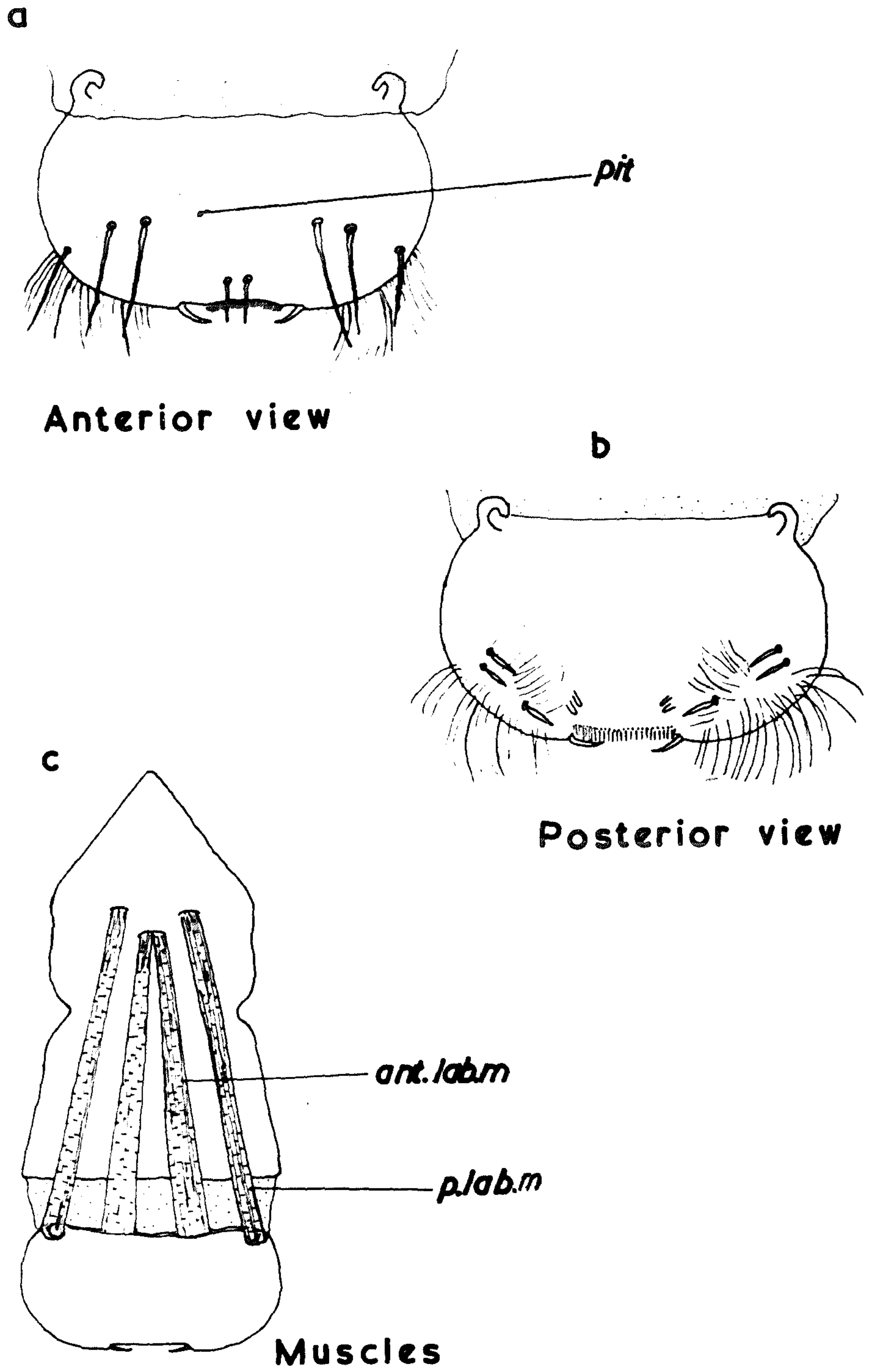
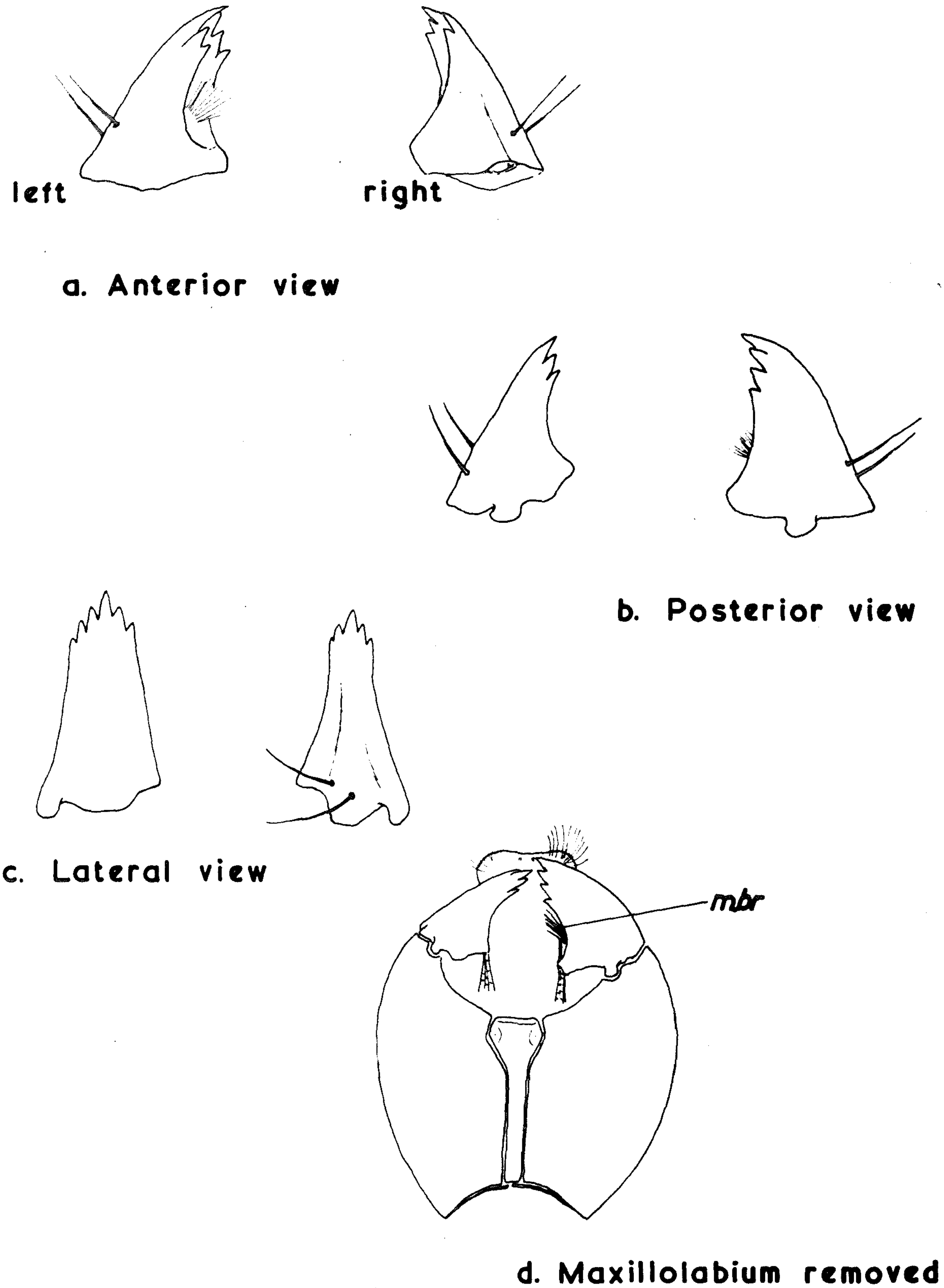

a
pit
Anterior view
b
Posterior view
c
ant.lab.m
p.lab.m
Muscles

Fig.3. Labrum.

considerable relation between feeding habits and the position of the eyes.

The mouthparts are mandibulate and the mandibles especially are well developed. The labrum is hinged to the fronto-clypeus by a membranous ante-clypeus, which is sometimes not readily seen. The labrum (Fig.3 lab.) is yellow brown in colour and is broader than long with a shallow excision on the anterior margin. On either side of the upper surface there is a row of three yellow spines. The two spines directly behind the median excision are not always complete in preparations. Slightly to the right of the median line is an unpaired pit which occurs constantly (Fig.3 pit.); no spine has been seen in it. On the anterior margin of the labrum are 4 curved short, stout spines, 2 on either side of the excision and directed inwards. Ventrally (Fig.3 ) on the lateral edges and extending slightly inwards is a brush-like arrangement of bristles together with a pair of spear shaped spines. Along the anterior edge between the two inwardly curved spines is a row of closely compacted short hairs. Behind this and on each side is a pair of protruberances. These bristles, spines and hairs are probably sensory in function and give also mechanical aid in feeding.

The mandibles are very heavily chitinised so that in the later instars they are dark brown in colour. They are asymmetrical, and the cutting surface is anterior, being composed of a variable number of teeth. When these are well developed, as in the last instar, there appear to be 6 teeth on the right and 4 on the left mandible. The third one from the dorsal edge in the right, and the second one from the dorsal edge in the




d. Maxillolabium removed to show position of mandibles

Fig.4. The mandibles.

left mandible are the longest. The left mandible (Fig. 4 a ) is stouter than the right and has a deep concavity in which is situated a bunch of bristles forming a brush. The right mandible is generally narrower anteriorly, in lateral view (Fig.4 b) and has fewer teeth. As with the other larvae of this family it lacks a brush. On the posterior convex surface of each mandible are situated two setae, ventrally there is a condyle which articulates with a facet on the anterior margin of the genae; the mandible also articulates dorsally by means of a similar joint, the edge of the genae being produced into a slight protruberance, which articulates with a concavity on the edge of the mandible.

The maxilla and labium are, as in all other Trichoptera, partially joined at their bases to form the maxillo-labium (Fig.5 ). The maxillary palp is five segmented, the distal segment bearing several rounded papillae or sense rods. The proximal segment of the palp has a large mesially directed sclerite and from its inner distal margin the inner lobe of the maxilla originates (Fig.5 i.l.). According to Das, this basal segment is definitely a segment of the palp and not a palpifer as indicated by Nielsen (1942). According to Macdonald, more recent authors are inclined to agree with Das' evidence: "The basal segment of

> the palp is often misinterpreted as the palpifer owing to its partial or complete fusion with the basal part of the inner lobe, but it can be definitely identified by the insertion of the palpal muscles upon its base".

The palpifer in this instance is a membranous area distal to the stipes. Following Das' inclusion of the proximal segment in the palp the number of segments is five. This however does not agree with

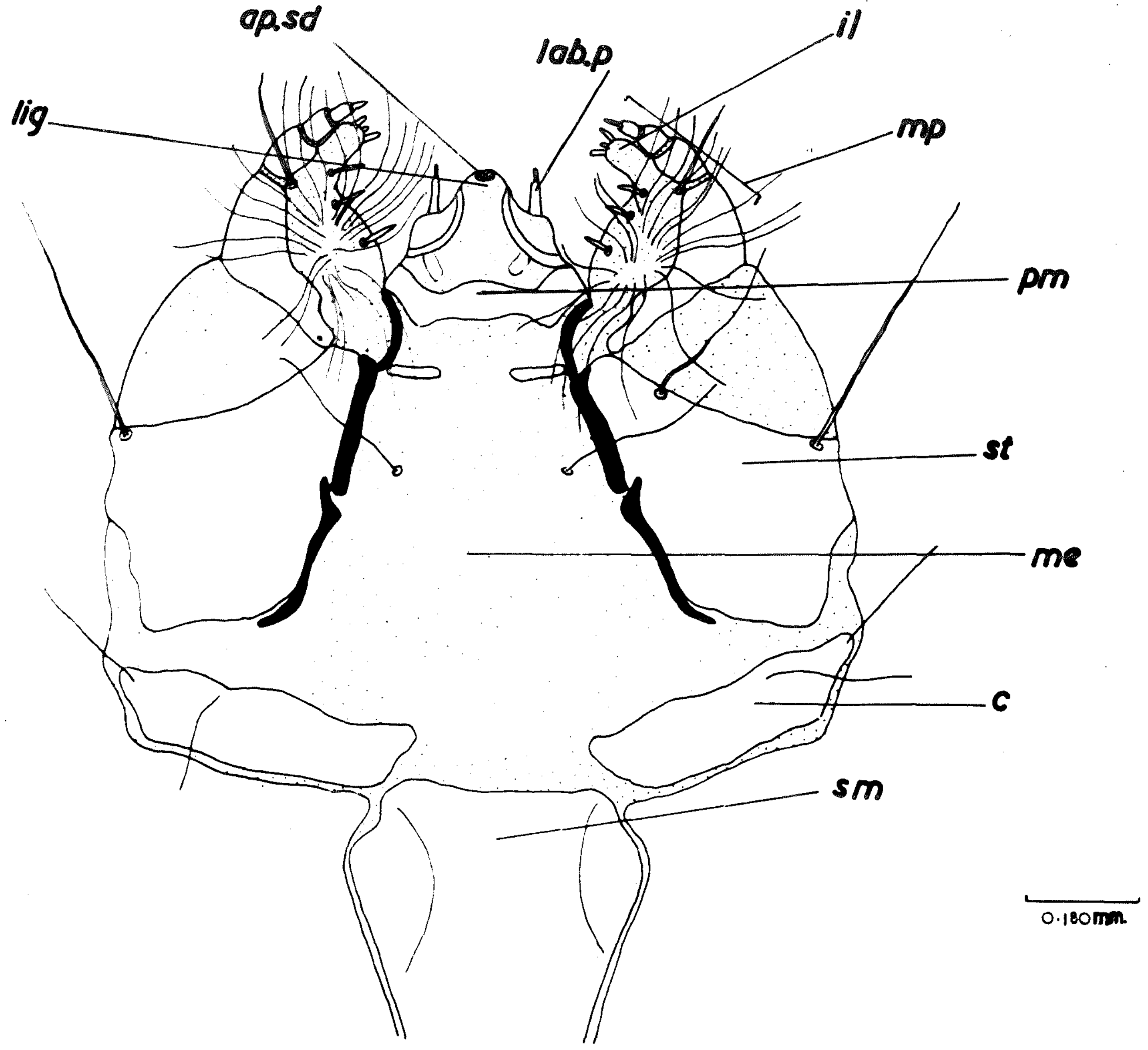



Fig. 5. Maxillolabium.

Orcutt (1934), who states that for the family Leptoceridae the number of segments is always one less than all other families in the order, no matter what the proximal segment is called. Hickin (1946) says that "In the Leptoceridae there are often only three segments apparent". He does not regard the 'palpifer' or basal segment as being part of the palp proper. He goes on to say that in some species of the family Leptoceridae the fourth or proximal segment of the palp is apparent as a sclerotised patch on the outer side near the base of the maxillary lobe. In T. obsoleta larvae it would seem that we have an exception as Hickin notes; for, even excluding the large proximal segment from the palp proper, the number of segments would be four and not three, as stated by Orcutt to be the number of segments in the maxillary palp of Leptoceridae. The inner lobe of the maxilla has been variously homologised, either as galea or lacinia or fused galea and lacinia. Das maintains that it can be definitely identified as lacinia on evidence produced by investigation of the musculature. Distally the lacinia bears sensory papillae, and spear shaped spines are distributed along the median margin. Proximally the lobe forms a bulbous area with a bunch of fine hairs radiating out all round (Fig.5 ). The stipes is a large sclerite (Fig.5 st.) bearing two large bristles on the anterior margin which makes an oblique angle with the basal segment of the palp, the membranous area between representing the palpifer. The cardo (Fig.5 c.) is much more heavily chitinised, and is in the form of a bar or rod with its long axis at right angles to that of the stipes; it also bears 2 bristles along the anterior margin.

The premental lobe of the labium (Das 1937) forms a terminal ligula carrying the opening of the silk duct, and on either side there is a one

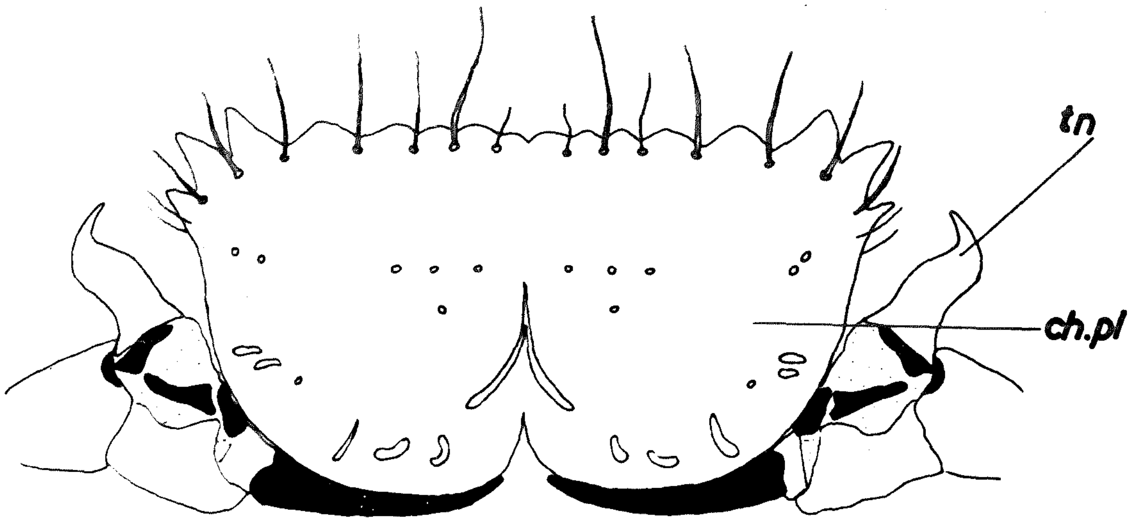


Fig. 6. Pronotum.

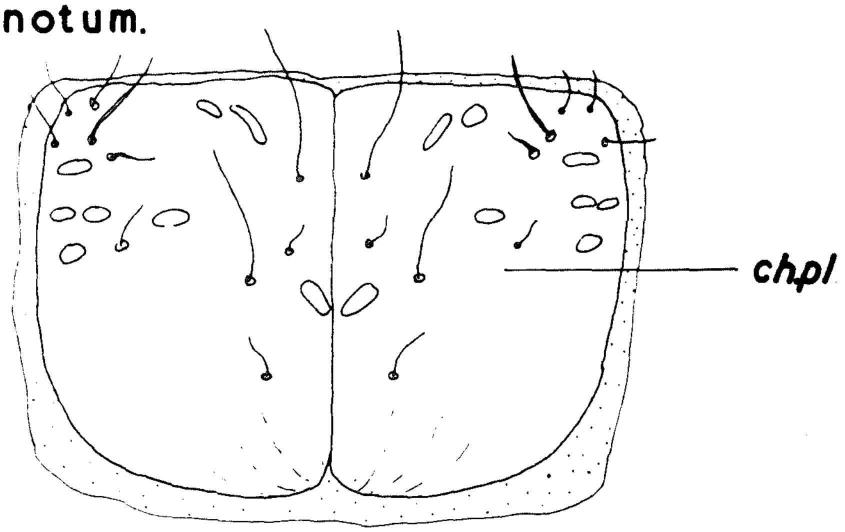


Fig. 7. Mesonotum.

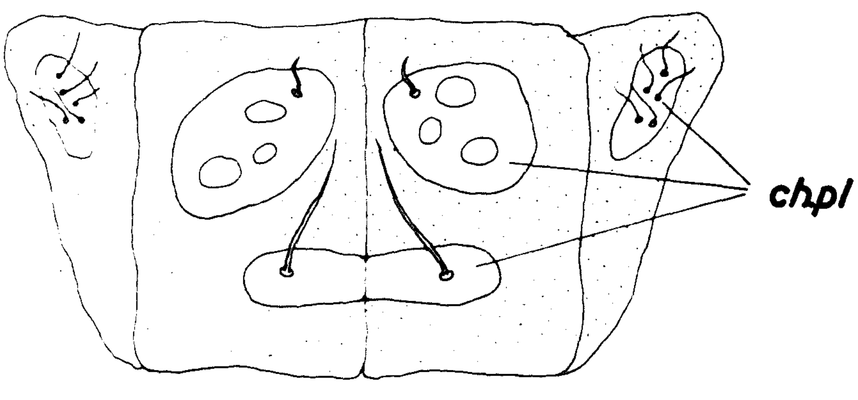


Fig. 8. Metanotum.

segmented palp each with two sense rods.(Fig.5 lig.). The labium is retractile and is directed a little posteriorly. As indicated earlier, Das' work points to the fact that the postmentum consists of a membranous mentum (Fig.5 me) and a chitinous plate, the submentum (Fig.5 sm.), lying distal to the origin of the premental muscles and the tentorial pits. As stated by Das, for all larval trichoptera, and so it was found on dissection of this species, no muscles arise or are inserted upon the postmentum. It is interesting to note that Siltala (1907) in Orcutt 1934, was of the opinion that the so called 'gula' was in fact the submentum in the families Hydroptilidae and Hydropsychidae.

The Thorax.

As in all other Leptoceridae the pronotum and mesonotum are chitinised while the metanotum is membranous. Each segment bears well developed legs. Usually the posterior portion of the head is retracted beneath the anterior margin of the pronotum. The pronotum (Fig.6) has a crenate anterior margin, with a single long seta originating from each indentation. There is a median longitudinal suture; the light areas on the pronotum form a characteristic pattern. The trochantin of the propleuron is produced forward to form a claw or hook (Fig.6 tn.) and is found in all the instars except the first. Ventrally the prothorax is entirely membranous and does not have a prosternal horn which frequently occurs in Leptocerid larvae, but there is an intersegmental chitinisation in the form of a convex narrow strip between the prothorax and mesothorax. There are no other areas of chitinisation nor are there any setae.

The mesonotum (Fig.7 ) is less heavily chitinised than the former;

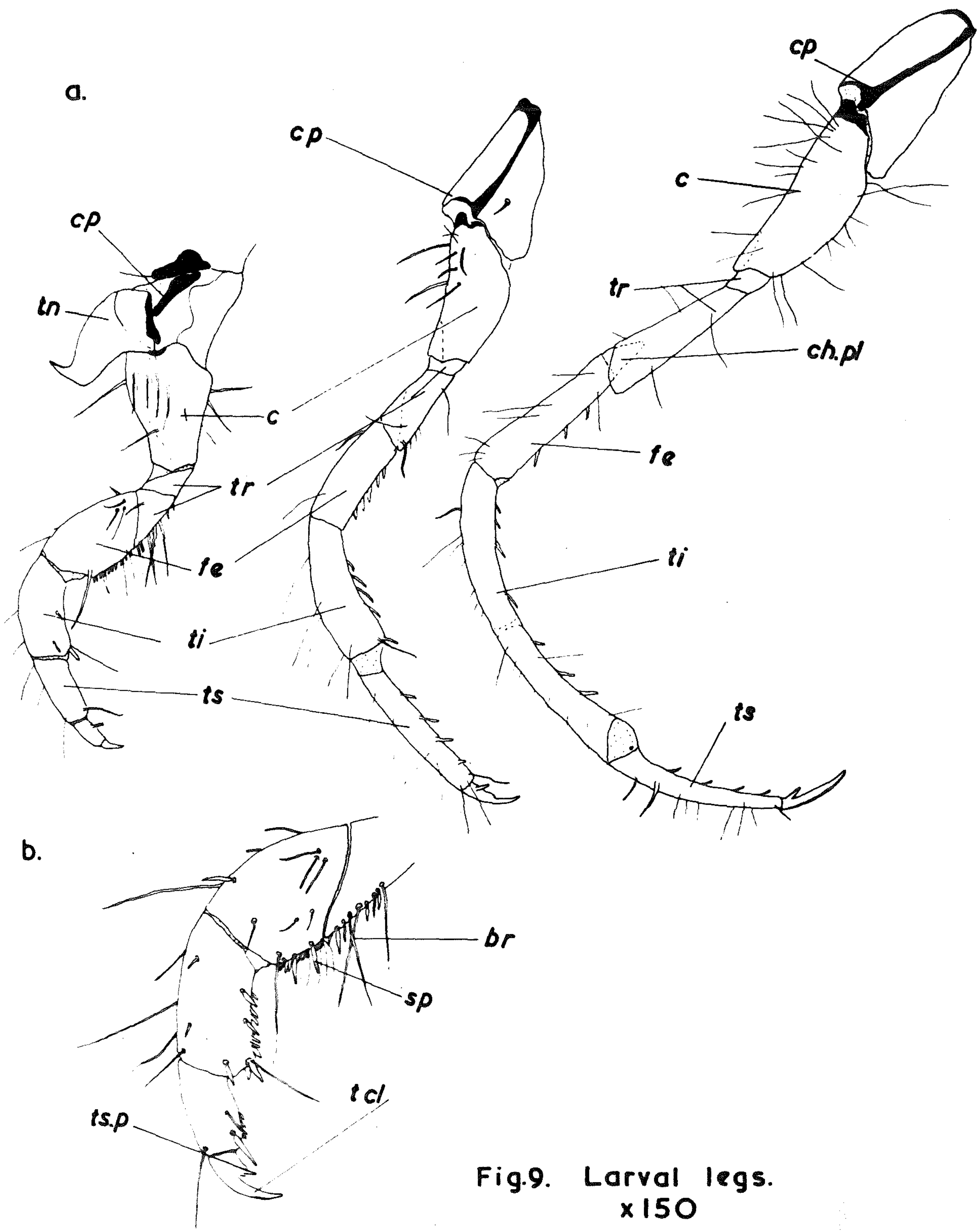



Fig.9. Larval legs.
x150

it is divided by a median longitudinal suture and has characteristic areas of light colour. Ventrally there is a chitinous sternopleurite at the base of each coxa; there are no setae. Scattered over the pronota and mesonota are black bristles arranged in a constant pattern.

The meta notum (Fig.8 )is membranous except for five small areas of chitinisation. Situated medianly are two sclerites which have the same shape as the mesonotal sclerites only they are about one quarter the size. Proximally there is a median sclerite which is partly or sometimes completely divided and carries two long setae. Frequently there is a small circular sclerite on each side. Laterally situated is an elongated sclerite with the long axis parallel to that of the body; this bears a varying number of setae. Ventrally the sternum is membranous but has several black bristles, which in the larger larvae are situated on small chitinous plates.

The legs are well developed (Fig. 9 a) and play an important part in the life of the larva. They are used for crawling around the stream bed amongst surface debris and 'pond weed'. The tarsal claws (Fig.9 b) provide an effective means whereby the larva holds itself on to the substrate against the force of the current. The coxopleurite (Fig.9a cp.) of the propleuron is small but well chitinised, and progressively larger in the meso and meta pleura. The prothoracic legs are short; they are used for holding pieces of vegetation during the process of case building, and while feeding. The mesothoracic legs are slightly longer than the prothoracic legs and are used for holding on to objects like the latter, but are used more for locomotion. The coxae of each leg of the first and second legs are held close together, each pair of legs articulates so that the legs of one pair oppose each other. The femur being held out, and slightly forward of the transverse plane, and the tibia and tarsus held inwards so that the lower half of the legs of both pairs enables them to be efficient graspers. The metathoracic legs are longer than the first two pairs and are the main means of locomotion. They are usually held at a different angle to the others: the trochanter and femur are directed forwards and slightly backwards, while the remaining portion is articulated so that the tarsal joint comes in contact with the substrate forward of the head. The metathoracic legs are frequently seen waving around feeling for objects in front and to the sides, contacting the surface they dig in the tarsal claws pulling forward the animal and case.

The ratio of leg lengths is $1:1\frac{1}{4}:2$, this being the average of the second to sixth instars. The ratio of each individual instar departing little from $1: 1\frac{1}{2}:2$, the middle leg varying slightly in each case. In order to obtain these length ratios the legs were drawn to scale with a squared eyepiece and measured from the drawings. The leg segments are armed with bristles and spines, the latter of which are found along the inner margins of the femur and tibia of the first and second legs, there being only a few on the third leg. On all three legs there is a spine at the base of the tarsal claw (Fig.9 b t.cl.). Also on the distal inner margin of the femur of the first leg there is a well developed stout bristle (Fig.9 b br). The probable function of these spines and bristles will be dealt with later. The tibia of the metathoracic leg has an

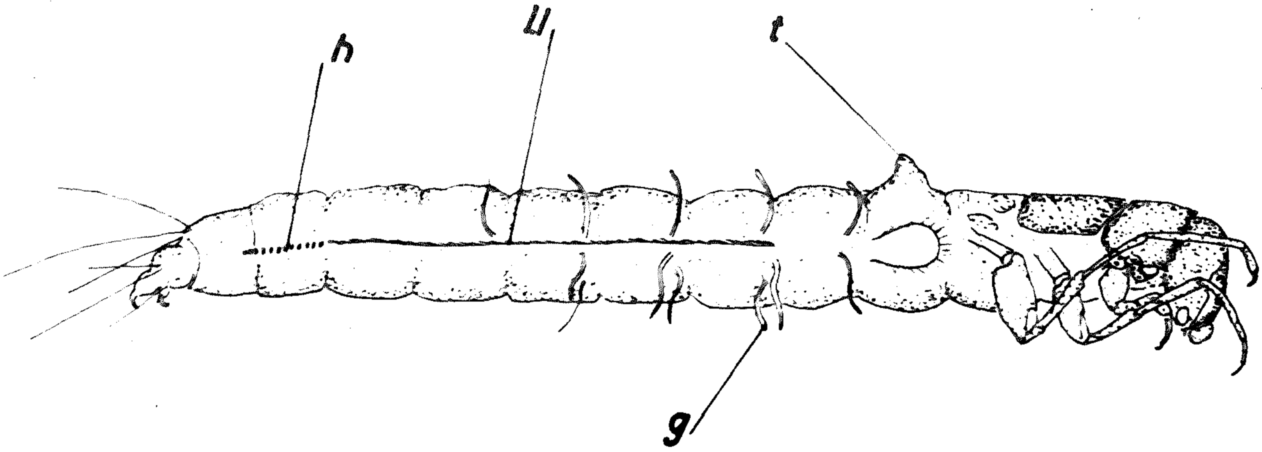



Fig.10. Lateral view of larva.

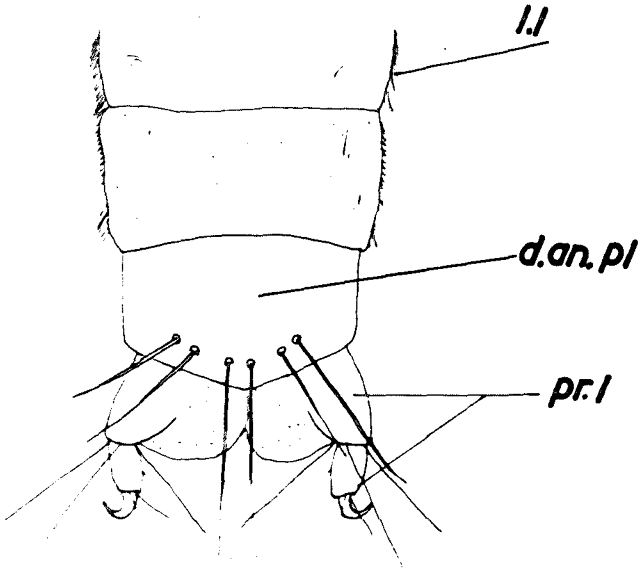



Fig.11. Dorsal aspect of ninth abdominal segment and prolegs.

incipient division into two but it is not a joint (Fig. 9 a). In the mesothoracic and metathoracic legs the membrane between the femur and trochanter has a small chitinous plate (Fig.9 a) which is inserted in a deep incision of the distal segment of the trochanter, thus movement is fairly limited between these two segments. A similar incision occurs on the distal margin of the coxa (Fig.9 a) of all legs so that the trochanter may move in an upward direction. The proximal segment of the trochanter is always small. The legs are marked with alternate bands of light and dark areas. Scattered over the dorsal surface of each segment are numerous black bristles.

The Abdomen.

The abdomen is cylindrical, narrowing slightly towards the posterior end, and the segments are clearly demarcated. It is almost entirely membranous and is cream in colour. The first abdominal segment bears three tubercles (Fig.10 t.): one dorsal, which is retractile, has a number of tracheole endings near the surface; the two lateral tubercles are also retractile and have the posterior surface slightly chitinised forming an oval plate with a fringe of hairs on the anterior margin, and a few bristles. The main function of these tubercles would appear to be to give some support to the larva in its case, holding the larva in the centre so that a current of water may flow evenly through the case. This current of water is drawn in by the undulating movements of the abdomen. The tubercles assist this movement by acting as an anchor from which the abdomen pivots. The rest of the abdomen is almost entirely devoid of any bristles except for the ninth segment. There is a lateral line or fringe of hairs (Fig.10 l.l.) which runs from the anterior edge of the third abdominal segment to the eighth. On this segment there is a line of small chitinous hooks (Fig.10 h.) at the beginning of the segment, which is followed by a continuation of the lateral line fringe of hairs.

Tracheal gills are of the filamentous type, and when present are on either side of the lateral line from segment three to segment eight (Fig.10 g.). There is a dorsal and a ventral row of gills and immediately below the lateral line there is a ventrolateral row, they are always situated on the anterior margin of the segment. Hudson (1904) states that for this larva, then known as Pseudonema obsoleta: "There are seven pairs of respitatory filaments on each side of the larva". This is incorrect as the number varies within each instar, and the total number increases with age, as will be seen in Sub-section VI.

The last abdominal segment is slightly narrower than the preceding ones and has a dorsal anal plate with three pairs of long black bristles and a pair of short ones (Fig.11). The first segment of each proleg (Fig.11 pr.1) is bulbous and has a chitinous plate dorsally which bears a row of four large bristles. The anus opens between the basal segment of each proleg. These segments are closely apposed and give the appearance of a tenth segment. The distal segment is incipiently divided and ends in a strong short hook (Fig.10). There are a number of bristles on this distal segment. The hooks are directed latero ventrally and serve as very efficient anchors in the silk lining of the larval case.

iv. The number and growth of larval instars.

Little work has been done on the growth of caddis larvae. Hanna (1957-59) has given some indication of the growth of several British species of caddis larvae by using millimetre length classes. By graphing the percentage of these in a given sample, against time, she was able to indicate the overall growth pattern of the larvae of different species over a period of approximately 2 years. Nielsen (1942) has given a fully detailed and illustrated account of the post-embryonic development, among other things, of caddisflies. Nielsen says of Dyars theory on the geometrical increase in size of sclerites at each ecdysis as applied by him to caddisflies, that when one is studying the development by using head width as the vital measurement, the resulting figures fall into well defined groups, whose mean values form a quotient series, these being equal to different larval stages or instar classes. These facts therefore discount Siltala's view that single larval stages are only differentiated by observation in an aquarium. Nielsen says that in the forms examined by him only in Sericostoma pedemontanum is the individual variation so great that the results become doubtful.

Wolfe (1949) recalls that Wesenberg-Lund (1913-14) discussed two methods that could be used for the study of the growth of insects and the determination of the size, structural changes and duration of successive instars; firstly a statistical analysis of the natural population in the field by sampling at regular intervals, and secondly by culturing from the egg in the laboratory in a well balanced aquarium. Relatively constant temperatures and adequate food are important for the achivement

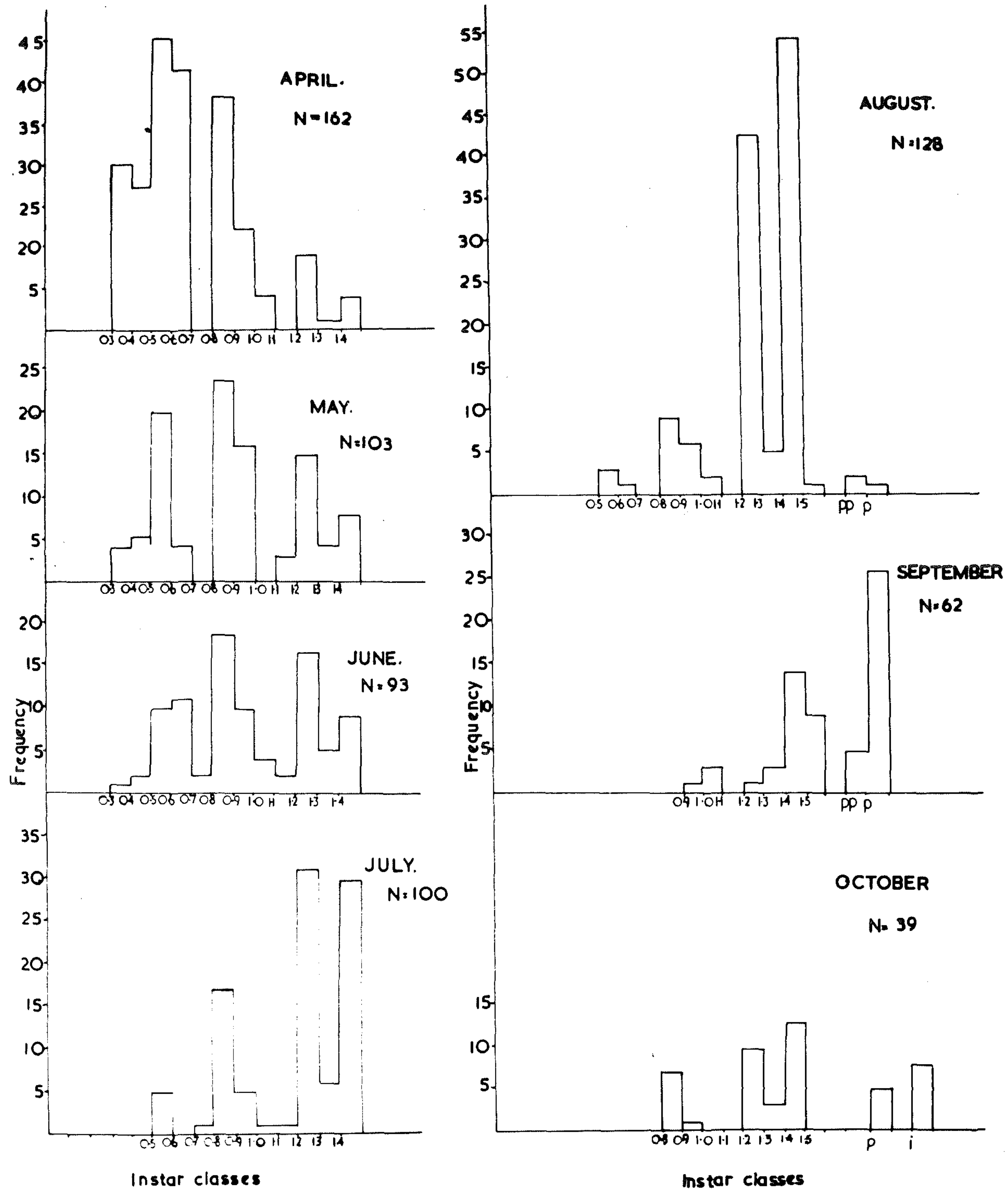



Table I. To show the change in proportion of the instar classes from April until October. pp. pharate pupae: p. pupae: i. imago.

of consistent results. "The first method should treated with care give the most accurate picture in direct relation to the environment. However under accurately defined conditions the second method gives dependable results" (Wesenberg-Lund) as quoted by Wolfe (1949). Corbet (1957) in the section on larval development in his study of the life history of the Emperor Dragonfly *Anax imperator*, studied the growth and distribution of the larvae by making length frequency analyses of samples taken from the population at regular intervals. Corbet's results confirm the first of the above statements of Wesenberg-Lund's.

Because of the different degree of contracion of the soft abdominal segments, when the larvae were killed in 70% alcohol, it was realised that length frequency analyses would not by any means be consistent, and for more accurate comparative work other than just obtaining the overall growth of the larvae as shown by Hanna (1957-59) a more concise method would have to be used for this species. Following Nielsen's proof of Dyar's work, head widths were measured for each sample. It was from head width frequency graphs that the larvae fell clearly into five groups (Table 1 for April), and these same groups were repeated, if present, in each successive sample. These groups were later recognised as definitely being equivalent to instar classes. Thus having found a reliable method whereby the individuals in a sample could be classified, an analysis of the natural population could be shown, on a valid comparative basis, for each month. This was done by constructing histograms and because each group represents an instar class an idea of the duration of larval life, together with the number of instars was obtained.

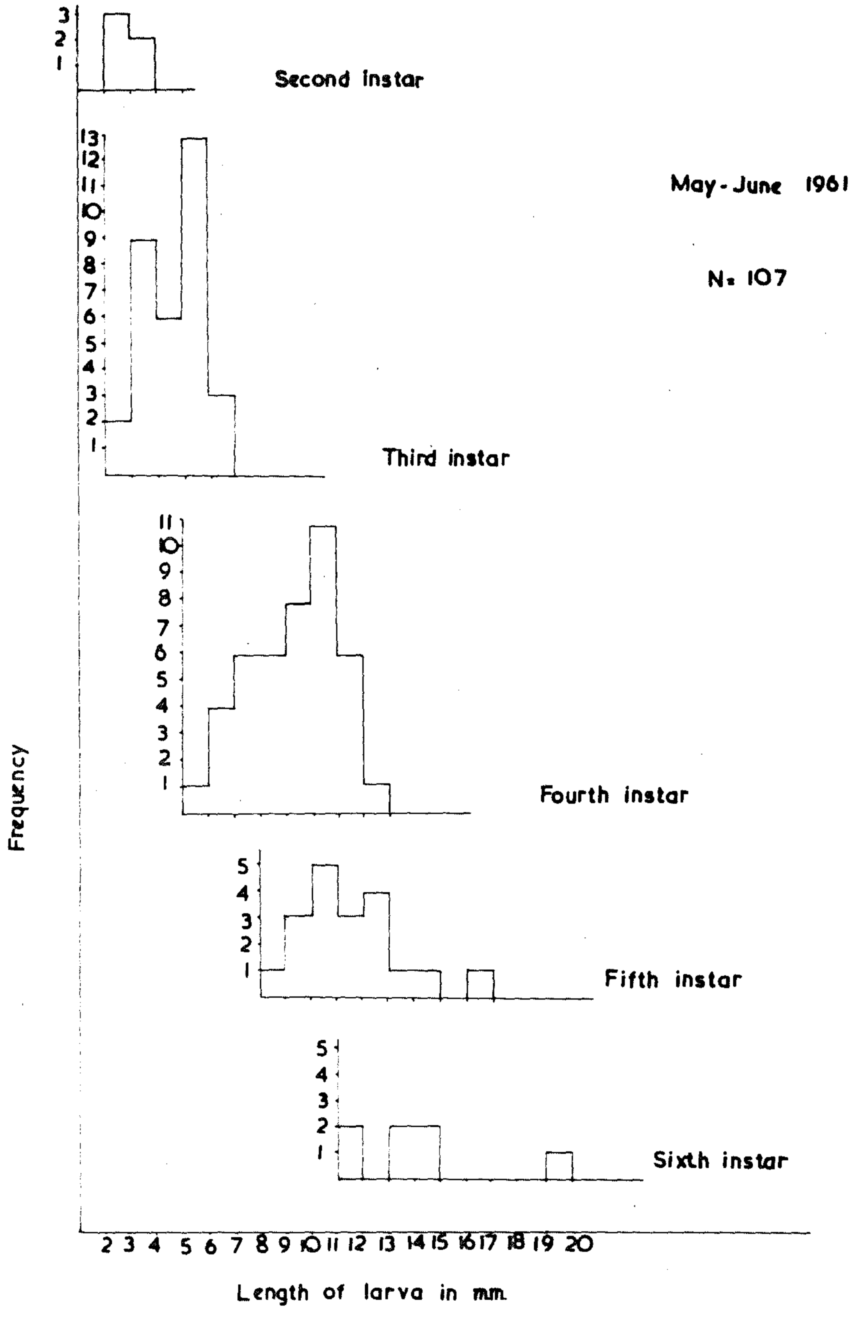



Table 2. Length of larvae for each instar.

The following are the approximate total length and head width measurements for the instar classes present in the samples. The 1st instar was not collected by the methods used for this particular work and is not represented in the graphs or tables.

| | | |
|---|---|---|
| 2nd instar | Head width = | .4mm |
| | Length = | 2 - 4mm |
| 3rd instar | Head width = | .4 - .7mm |
| | Length = | 3.5 - 7mm |
| 4th instar | Head width = | .7 - 1.1mm |
| | Length = | 6.5 - 13mm |
| 5th instar | Head width = | 1.1 - 1.3mm |
| | Length = | 9.5 - 17mm |
| 6th instar | Head width = | 1.3 - 1.5mm |
| | Length = | 10-11 - 21mm |

It can be seen from Table 2 that there is a considerable range of length in any one instar. The length is seen to increase with the age of the individual instar, as seen in the 3rd and 4th instar graphs; there is a marked drop after a certain peak in length is reached, presumably indicating ecdysis. The length of larvae in one instar class also overlaps, to a greater or lesser extent, that of the instar class before or after it. This variation in length within an instar is almost entirely due to the nature of the exoskeleton. The abdomen is soft, and its size depends largely upon the amount of food eaten and consequently upon the amount of fat body which has been laid down in the larva, especially in the last instar.

From the results graphed in Table 1 it can be seen that from April to July there is a distinct change in the frequency of individuals in each

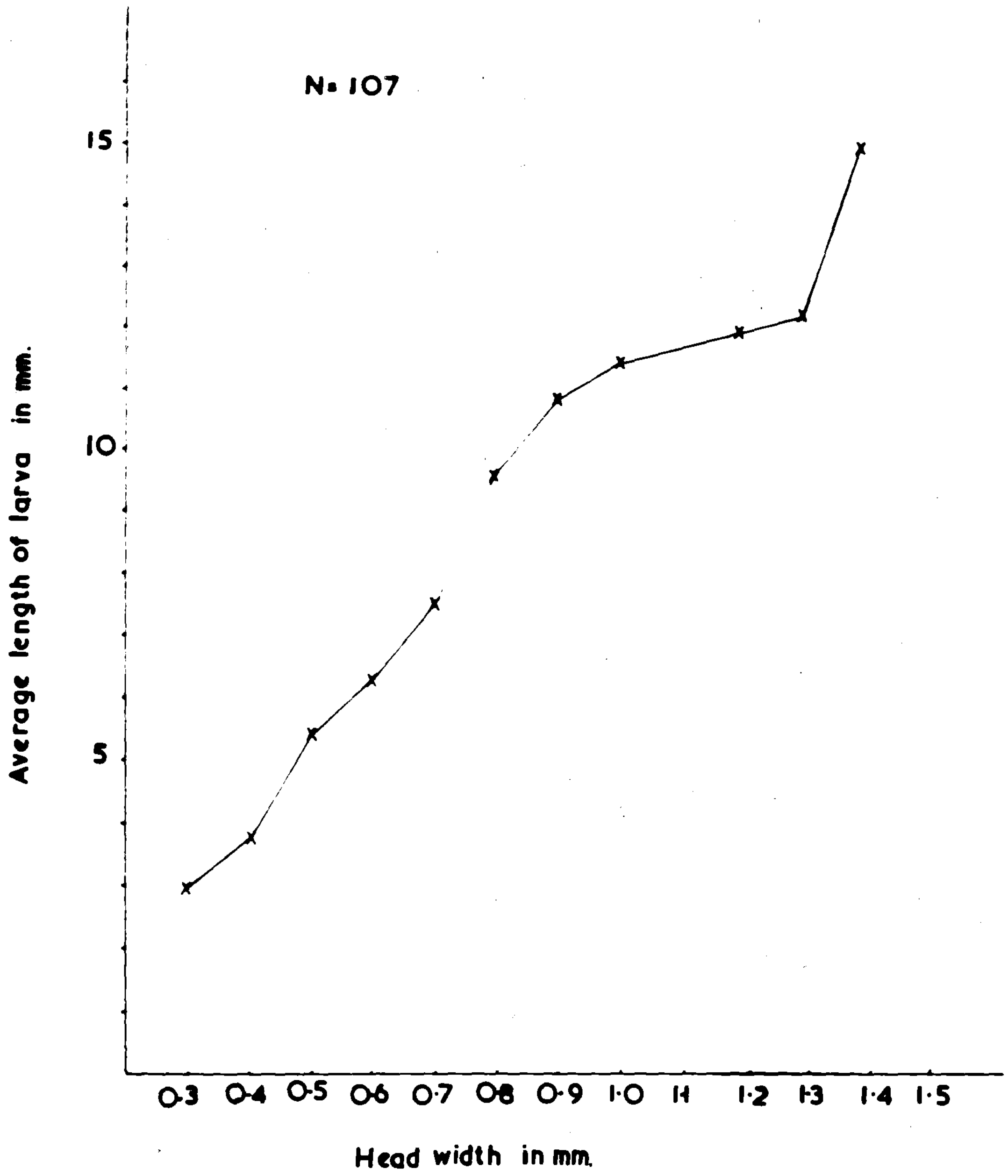



Fig.12. Growth of larva with age.

instar class. By July there were no 2nd instars and only a few 3rd instars in the sample, and the number of 5th and 6th instars had considerably increased when compared with the April sample. This change increases in August when two pharate pupae and one pupa were collected. By the end of September when only 62 larvae were taken,most of the larvae were in the 5th and 6th instar stage.

It would seem very likely from field observations early in the year and from the samples taken since April that there is an annual life cycle, most of the larvae in the samples having hatched the preceding summer and the majority of which will pupate during the summer following. That this might be so is indicated by the obvious paucity of 5th and 6th instars by April when it is still only the young instars which are more frequent in a sample. The growth of the larva does not decrease during the winter months, rather there is a steady increase in the overall growth pattern until the final larval stage is reached. This has not been noted for any individual larva, but can readily be deduced from Table 1.

When the average length of larvae in a sample was plotted against head width (Fig.12 ) it was found, as one would expect, the result approximates a straight line. There is an increase in length throughout the 2nd, 3rd, and 4th stages, but between the 4th and 5th stages there is a flattening of the curve until the 6th stage where there is a sharp increas e in length which appears fairly typical and may be correlated with the greater amount of fat body which is laid down in the abdomen at this time. The abdomen is no longer a semi-transparent cream colour, but is a dense creamy yellow, due to the colour of the fat body which is yellow.

v. <u>The duration of larval stages and the total larval period.</u>

While a fairly clear cut picture has been obtained concerning the overall larval growth during the year, the question of time involved for each instar and the the total larval period has proved to be difficult. This is firstly due to the fact that samples have been taken only from April 1961 onwards by which time the larval stages 2 - 6 were present, and secondly to the inadequate results obtained when Wesenberg-Lund's second method for calculating the duration and number of instars was used.

| Jan. | Feb. | March | April | May | June | July | Aug. | Sept. | Oct. | Nov. | Dec. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Adults | Adults | Adults | | | | | | | | Adults | Adults |
| Eggs | | | | | | | | | | | |
| 2<br>Larvae | | | 2,3,4, 5,6. | 2,3,4, 5,6. | 2,3,4, 5,6. | 3,4, 5,6. | 3,4, 5,6. | 4,5, 6. | 4,5, 6. | 4,5, 6. | 5,6. |
| Pupae | Pupae | Pupae | | | | | | Pupae | Pupae | Pupae | Pupae |

Table 3. Plan of Life Cycle. Nos. refer to instars.

It may be deduced from the table above that the approximate length of larval life will be a minimum of about 8 months. Because of the small sequence of samples and the limited time of observation from the field this can only be an approximate estimation. The maximum time may of course be more than this, depending on the time of hatching. A larva hatching in the early summer may have a larval stage of up to 9 or 10 months.

An attempt was made to give more evidence as to the total length of larval life and that of each instar. This was done by isolating specimens in floats in running water aquaria.

When moulting, the old exuvium splits along the frontal suture and along the median dorsal suture of the thoracic nota. After being cast it is wafted to the posterior end of the case by the undulating movements of the abdomen, gradually the exuvium is expelled through the caudal opening of the case. Because of this, it is difficult to detect ecdysis, without in some way damaging the case, and continued disturbance decreased the chances of survival of the larva so that the total number of moults could not be recorded. If it were easy to measure the larval head widths accurately while alive, part of the problem would be overcome as it would then only be necessary to rear the larvae for a short length of time. However, it is too difficult to get the larva in a satisfactory position for measuring without causing a deleterious effect. It was hoped that by rearing the larvae in the 1mm mesh floats that the exuvium would be caught up in it, so that moults could in this way be counted. However it was found that by the time the exuvium had been expelled from the caudal opening it was usually very broken up. The separate parts being small enough, they soon fell through the mesh. This is apparently what happened in a number of cases where larvae were kept for just over two months. The larvae used were 3rd and 4th instars, and by 2½ months all were dead or had escaped. Had the larvae been kept in larger containers they may have lived longer, the confined space probably having an effect on the mortality. Finally when larvae were found moulting, frequently the larvae had fixed the case to a solid substrate so that moulting proceeds undisturbed (this is not always the case, as larvae in the process of moulting may be found quite free on the stream bed); they were placed separately in small running water aquaria, so that they could be timed accurately from one moult to the next. These did not have much more success than the previous examples, individual larvae were reared for at the most 2 months, only in one instance was a further ecdysis recorded. These experiments were undertaken during the months of April, May and June, which is not apparently a particularly quiescent period of growth (Table 1.) so that in all probability the length of time involved in each larval instar from the 2nd onwards is approximately $1\frac{1}{2}$ - 2 months, the last, 6th instar, las ting longer than this. If the 6th instar stage is reached during the winter, then the time involved could be 3 - $3\frac{1}{2}$ months, as indicated by Table 2, and by one specimen kept in the laboratory.

In conclusion it may be said that in the time available it has been the analysis of the samples of the natural population which has proved to give the most informative results concerning the duration of larval stages, which, as suggested by Wesenberg-Lund in his methods outlined for these investigations, if treated with care can give reliable and accurate results.

vi. Structural changes occuring at ecdysis.

As previously mentioned, there are few definite structural features which change with each instar, and consequently head width measurements have been used to divide the larvae into instar classes. Having done this some aspects of the larval exoskeleton were then examined in order that extra proof of the validity of these instars may be obtained. The first instar, as already indicated, has not been found.

The 2nd instar larvae may be distinguished from the 3rd instar by the difference in the pronotum, which in the case of the latter, the anterior margin of each sclerite has crenations together with 6 black bristles

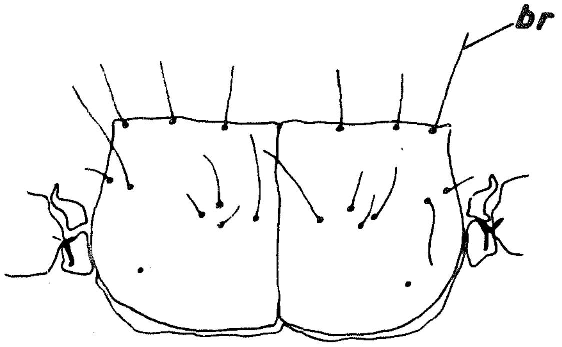



Fig.13. Pronotum – second instar.

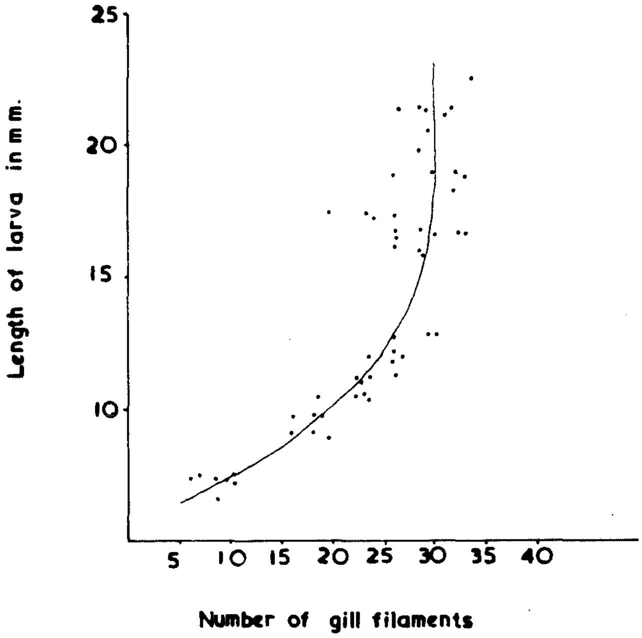



Fig.14. Increase in number of gills with length of larva.

(Fig.6 br.). In the 2nd instar the pronotum lacks these crenations and has only 3 bristles on either side (Fig.13 br). The mesonotum in both these instars is only slightly chitinised compared with the later instars. The degree of chitinisation of all sclerites is greater in the later instars, indica ting that probably these stages have a longer duration than the 2nd and 3rd instars.

Gill counts from the 2nd to 6th instars show that there is a gradual increase in the total number of gills with the age of the instar. Each instar exhibits a range in number of gills present, no number being constant for any instar. The number of gills as pointed out by Dodds and Hisaw (1925) is fairly well correlated with length of the larva; there was found in this case to be a similar correlation as seen in Fig.14.

Lestage (1921) gives Siltala's scheme for the disposition of gills along the abdomen.

| | D | L | | V |
|---|---|---|---|---|
| 1 | | | | |
| ii | 1 | | | 1 |
| iii | 1 | | 1 | 1 |
| iv | 1 | | 1 | 1 |
| v | 1 | | 1 | 1 |
| vi | 1 | | 1 | 1 |
| vii | | | | 1 |
| viii | | | | 1 |
| ix | | | | |

Formula = 5 + 0 +4 + 7 = 16

Thus the above formula may be used plus the total number. This formula varies from that used by Siltala in that he uses the number of gills per segment as the units of the formula, the resulting formula is therefore longer than that used here. This consists of three parts, the dorsal, latero-ventral and ventral rows of gills on one side, the total

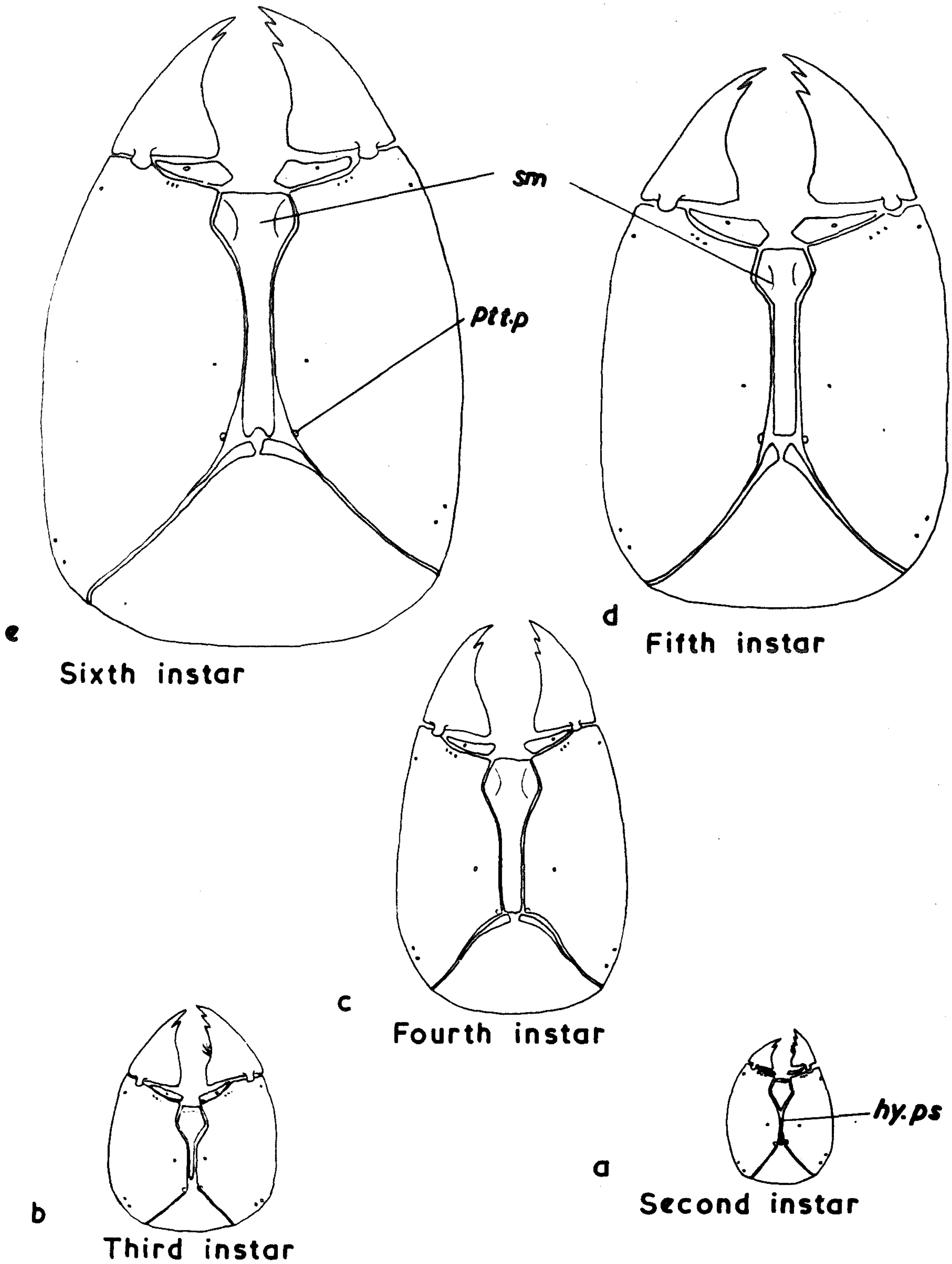



Fig.15. Ventral view of larval heads to show development of the submental sclerite.

number of gills being double the formula given. The following is a table showing the ranges of gill counts for each instar. The total number at the bottom or top of the range in each instar overlaps the one preceding it.

| 2nd instar | 3rd instar | 4th instar | 5th instar | 6th instar |
|---|---|---|---|---|
| 1+0+0+1=2 | 3+0+0+2=5 | 3+0+3+3=9 | 4+0+3+4=11 | 5+0+3+7=15 |
| 1+0+0+2=3 | 3+0+0+3=6 | 4+0+2+4=10 | 4+0+2+6=12 | 5+0+3+6=14 |
| 2+0+0+3=5 | 3+0+1+2=6 | 5+0+1+5=11 | 5+0+3+5=13 | 6+0+4+7=17 |
| 2+0+1+2=5 | 3+0+2+2=7 | 5+0+3+5=13 | 5+0+3+6=14 | 6+0+6+6=18 |
| | 3+0+2+3=8 | 5+0+4+4=13 | 5+0+4+5=14 | |
| | 4+0+1+3=8 | | 5+0+3+7=15 | |
| | 4+0+2+2=8 | | 5+0+4+6=15 | |
| | 4+0+1+4=9 | | 5+0+4+7=16 | |
| | 4+0+2+3=9 | | 5+0+5+6=16 | |
| | 4+0+2+4=10 | | 6+0+6+6=18 | |
| | 5+0+3+3=11 | | | |

Table 4. To show the range of gill formulae in each instar. This was obtained from a random sample.

The submentum (gula of some) is the only sclerite which shows any marked change from one instar to the next: this is more noticeable in the early instars. In the 2nd instar this sclerite is in the form of a triangle, the apex of the triangle diminishing in size to a fine point in the hypostomal suture (Fig.15 a ). The 3rd instar shows the sclerite extending posteriorly in a narrow line (Fig.15 b ). The 4th instar

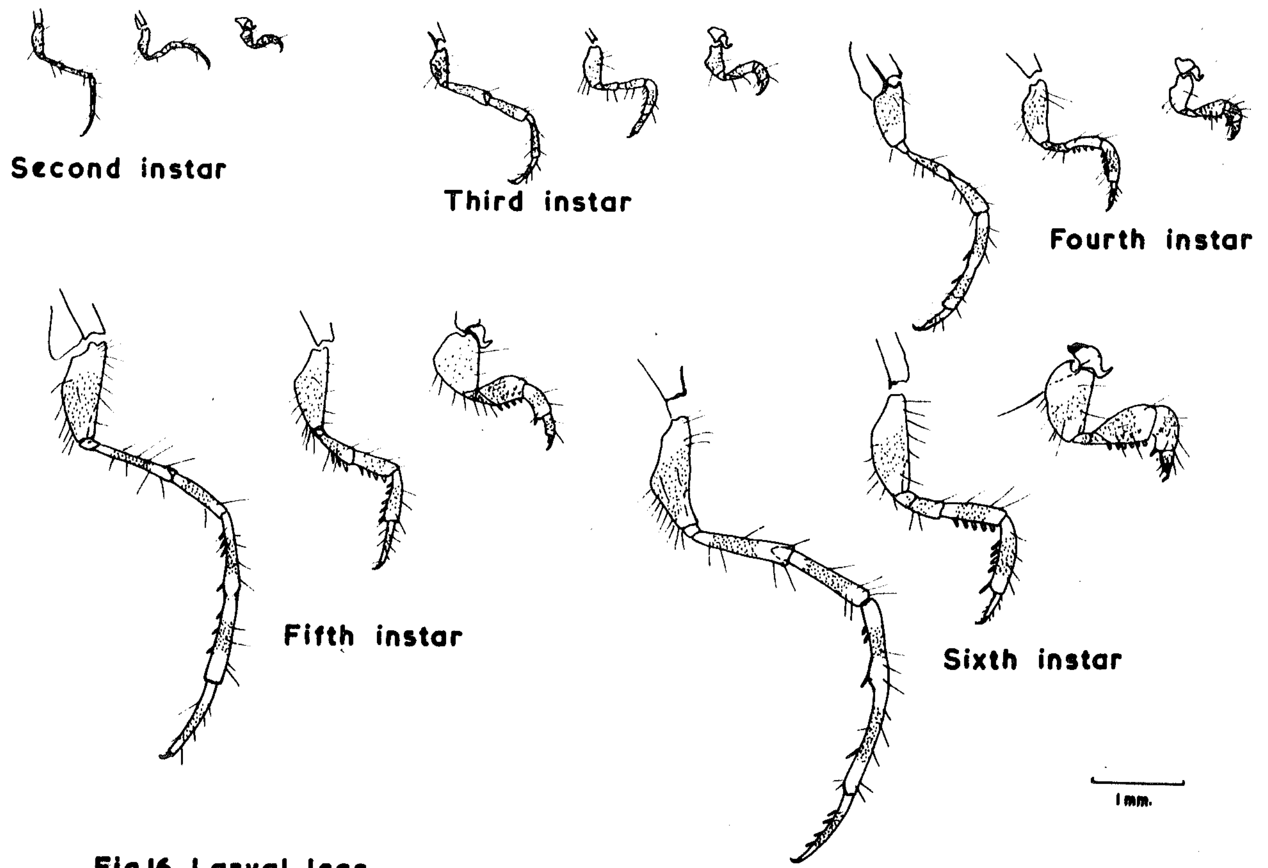



Fig.16. Larval legs.

shows this posterior portion slightly thicker and also there is a chitinisation along the median ends of the post-occiput, which is seen as distinct wedge-shaped bars (Fig.15 c ). In the 5th and 6th instars the submentum is fairly similar in shape. In the 6th instar the posterior part is better developed and the posterior margin has a distinct notch on it (Fig.15 e). In both these instars the median ends of the post-occiput are well developed (Fig.15 d and Fig.15 e).

There is a progressive increase in the length of the legs with each instar, but the ratio of pro., mesa., and meta legs always remains the same i.e. $1:1\frac{1}{4}:2$. Also the form of the segments and the development of bristles and spines are very similar in each instar. Fig.16 shows the legs drawn to scale for each instar. Below is a table giving the proportions of the leg segments for each instar.

| | 2nd instar | 3rd instar | 4th instar | 5th instar | 6th instar |
|---|---|---|---|---|---|
| | c. trfe. titar. | c. trfetitar | ctrfetitar | ctrfetitar | ctrfetitar |
| Pro. | 1+1+1+.5+.5=4 | 1+1+1+1+1=5 | 2+1+2+1+1=7 | 2+2+2+2+1=9 | 5+2*5+2+1=13 |
| Mesa | 1+1+1+1+1=5 | 2+1+1+2+1=7 | 2+1+2+2+1=8 | 2+1+2+2+1=8 | 5+2+3+5+2=17 |
| Meta | 1+2+2+2+1=8 | 2+2+2+2+1=9 | 3+3+2+5+1=14 | 3+3+2+5+1=14 | 5+5+4+9+4=27 |
| | =17 | =21 | =29 | =31 | =57 |

Table 5. Showing the proportions of leg segments in each instar.

From this it can be seen that the length of the segments generally increases in proportion, except for the last instar where the coxa in all three legs increases more than the other segments. Also it is the tibia which increases more than the other segments in the 4th, 5th and 6th

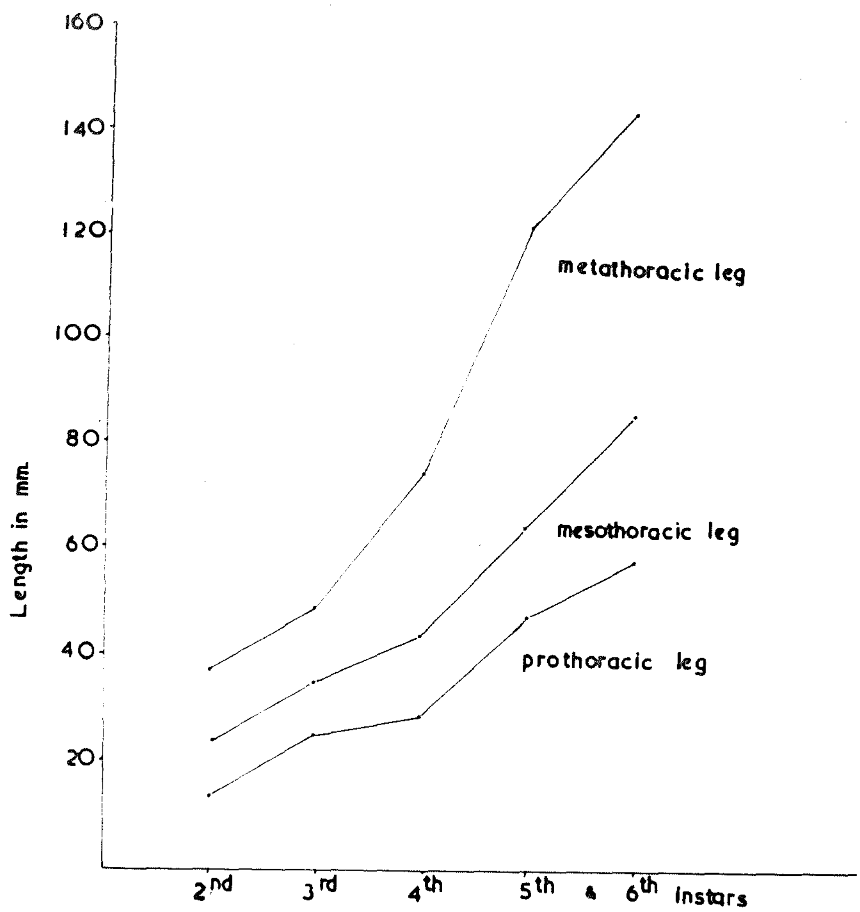



Fig.17. Increase in total length of larvae with age.

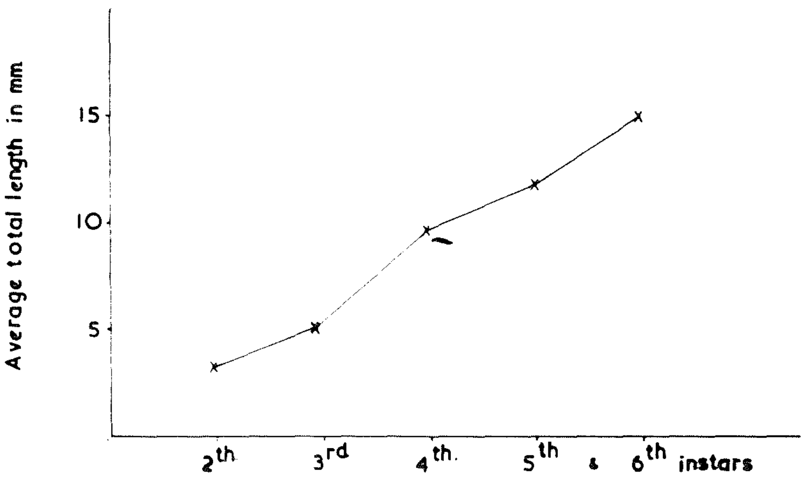



Fig.18. Average increase in total length per instar.

instars. Fig.17 shows the increase in total leg length with each instar, and is comparable with a graph showing the average increase in total larval length per instar (Fig.18).

The chaetotaxy of the labrum, frontoclypeus and the dorsal anal plate remain constant from the 2nd to the 6th instar. Except for the anterior margin of the pronotum already mentioned the remaining chaetotaxy was not examined in detail. The mandibles as they increase in size with successive instars show an increase in the degree of chitinisation, which by the 5th and 6th instars enables the larvae to be more capable of boring into wood, a habit characteristic for these instars.

The main changes occuring with ecdysis are clearly those associated with growth processes, the primary structural features remaining constant throughout the larval stages.

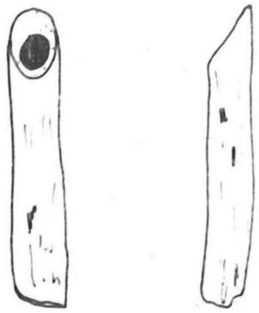
vii. Habitat distribution of the larval stages.

Merely from observation it was noted that larvae of all instars are distributed fairly uniformly throughout the stream. The most common places of habitation in the Upper Styx River were along the stream bed amongst the debris of wood and dead leaves, along the edges of the stream where the roots of trees and grasses enter the water and finally along the under surfaces of logs and smaller pieces of wood which happen to be floating in the water or leaning against the banks only partly submerged. As the season progresses changes take place which alter considerably the nature of the microhabitat of the larva. From late summer until the end of autumn, the surrounding vegetation, which is mostly deciduous, sheds a large amount of foliage into the stream, so that there is in some places a considerable thickness of leaves on the stream bed. From April to about the end of July samples taken from this microhabitat yield all instar classes, the majority being early instars (Table 1). During this time the young larvae, who construct their cases out of assorted bits of dead plant foliage, woody material and seeds, move about in the surface layer of debris obtaining their food and at the same time remaining camouflaged from predators.

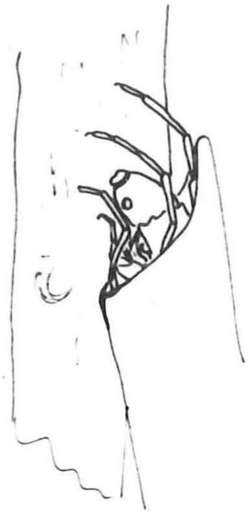
Later in the season the layer of leafy debris decreases, until there is nothing but sticks of all sizes littering the stream bed, which had been covered by the layer of leaves. By August a change takes place in the larval distribution. Samples of the stream bed no longer yield plentiful collections of larvae which by now are mostly of the 5th and 6th instar classes. After examination of the rest of the stream it was discovered that these larvae had migrated up the sides of the stream and on to any woody substrates submerged or partly submerged in the water. The usual sampling technique was therefore no longer used. Instead samples were obtained by hand picking all the larvae from the logs etc. until about 100 larvae were taken.

Correlated with this distinct change in microhabitat with the age of the individual, is the need for the last instar larvae to reach a solid object prior to pupation, this is nearly always some type of sodden wood, seldom has pupation been noted to take place in the old case of the last instar.

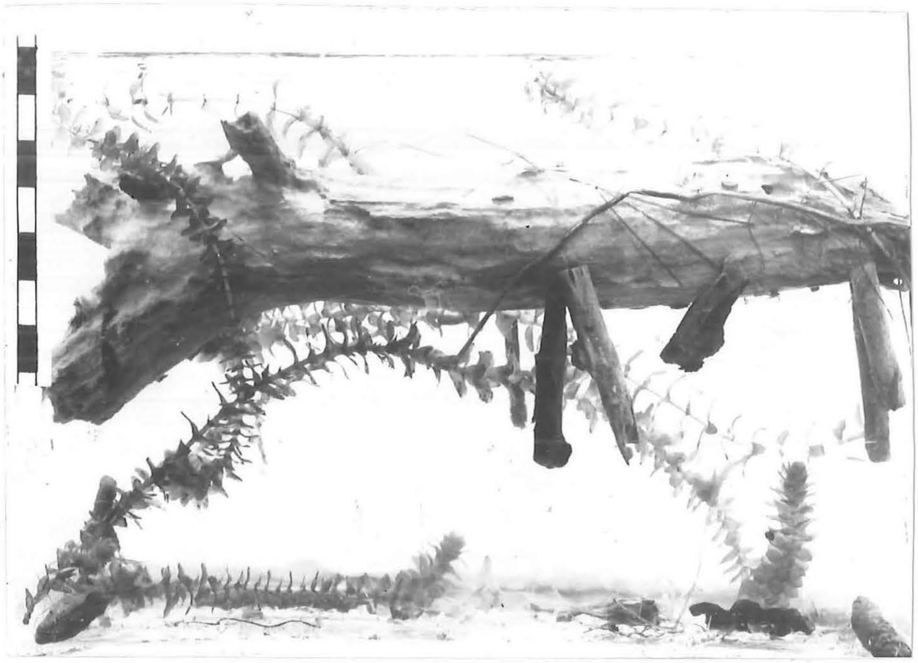
The larvae move readily along surfaces of logs clinging on with their tarsal claws and on occasions digging their mandibles in to add strength



a. Larval cases of hollowed twig type.



b. Larva in feeding position.




c. Larvae on submerged wood.

Fig.19.

to the grasp if needed. To enable this grasp to be more efficient and also to let the larva have protection at the same time as freedom of movement, the anterior end is fashioned in a particular way which appears constant in the larvae in hollowed wooden cases. Instead of being straight across the end it is fashioned by the larva in a dorsal ventral oblique angle (Fig.19 a ) so that when the larva pulls hard on to the substrate the case is held closely over the head of the larva and it is at an angle of about 45$^{\circ}$ to the substrate. Also the larva may crawl readily up the sides of objects while the head is still protected by the dorsal projection of the case, and the larva ia able to continue walking around and feeding on the surface of the wood quite readily (Fig.19 b ). When the larva is disturbed too much it loses its hold, contracts inside the case and drops to the stream bed so that to all appearances there is only a broken piece of wood being wafted along by the current. If the larva comes to rest in a suitable place, legs and head emerge and it crawls with a jerky motion to another suitable feeding substrate, under logs or on pond weed, Elodea, which is the frequent site of habitation in the Lower Styx River. Here the stream bed was too deep for examination. Figure 19 c gives an idea of the habitat of the larva beneath submerged logs. In the River Hawdon, Cass, where the population seems more dense, many of the larvae of 5th and 6th instars mainly, are found on the under surfaces of the numerous logs lying in the water, where also is the site of pupation.

To conclude this section it has been confirmed by the sampling techniques used that the young larvae predominate in the debris of the stream bed and as the larvae approach the 6th instar there is a shift in the habitat which is correlated with the time and site for pupation. This begins in late August and is in full swing by the end of September.

## 1/2 Pupation and the Pupal Stage

### i. Introduction.

Pupation in this species is rather an interesting study, but unfortunately material has only recently been available in the field so that work on this aspect of the life cycle has been fairly limited. Because of the activity of the larva prior to pupation, it was not until this project was in its final stages that the characteristic site for pupation was discovered.

Pupae were fixed in Carnoy and preserved in 70% alcohol. Exuviae were dehydrated and mounted in balsam for work on exoskeletal features. Some material was kept alive in running water aquaria for observation on the duration of the pupal stage.

### ii. Pupation as observed in the Field.

As mentioned in the previous section, 5th and 6th stage larvae migrate to substrates of a woody nature prior to pupation. It was originally thought, as indicated by Hudson (1904), that larvae in the last instar, when ready to pupate, made fast their case to the substrate with silk thread, and then sealed off the ends to form the pupal cell or cocoon, as commonly found in other caddisflies. It was frequently observed during August and September that when cases were pulled off logs, the larva had been in the process of burrowing into the substrate; this behaviour was so common it was thought that the larvae may have been obtaining food. Some cases, however, lacked larvae altogether, just empty cases affixed to the wood. It is this feature which has obviously led previous investigators to assume that pupation had been completed and the pharate imago

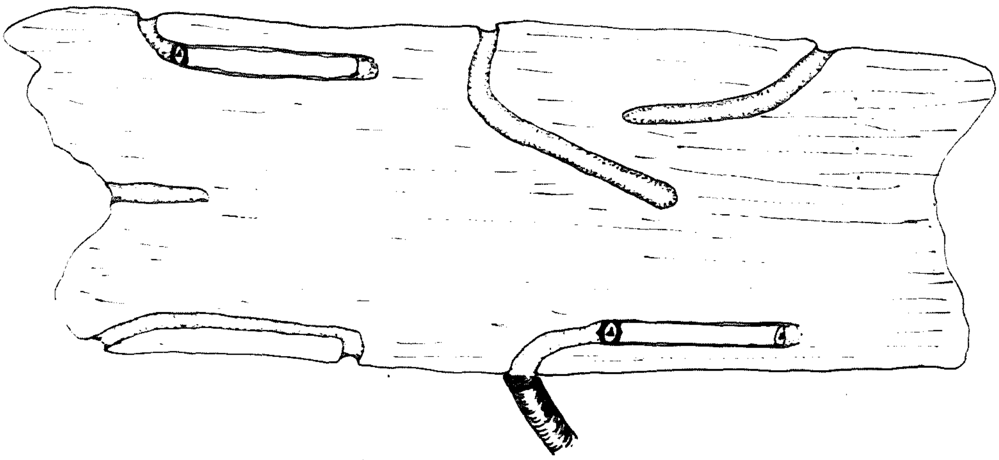


Fig.20. Pupal cells in sodden wood.

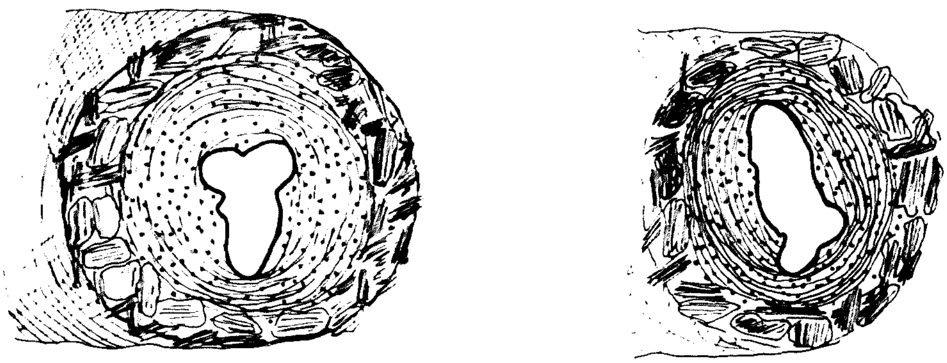


Fig.21. Anterior (left) and posterior membranes of a pupal cell.

had escaped. Upon further examination of the area from which these cases had been detacched it was found that directly opposite the cephalic opening of the larval case, a small tunnel big enough to accomodate the width of the larva led directly inwards. After paring away the wood fibres, the tunnel was found to end in a cross wall behind which was located the true pupal cocoon containing the prepupa, the pupa or the pharate imago. Many similar pupal cocoons were found in logs of sodden wood. More frequently the case had become detached and the pupae could only be located by systematically excavating the outer softer woody fibres of logs or sticks.

Some of these pupation sites were riddled with tunnels, many of which contained pupal cells. Sometimes the tunnel was hardly developed, the pupal cell being close to the surface; at other times the tunnel was as much as an inch long before the pupal cell began. The pupal cell nearly always lies parallel to the surface. Figure 20 shows a solid piece of wood containing some pupal cells exposed to view. As mentioned above, pupation in the old larval case is fairly uncommon, probably being resorted to when there is a scarcity of debris of a more solid nature. An example of this would be in the Lower Styx River. However, in the Upper Styx and in the Hawdon River, Cass, pupation generally takes place in sodden wood, which in these two areas is very plentiful.

When the larva has excavated the tunnel prior to pupation it may cut the silk threads holding the larval case to the substrate or this may remain attached until knocked off by some other means. Inside the tunnel the larva nearly always makes another contact with the surface so that a through current of water will finally be created in the pupal cocoon. Next the larva lines the tunnel with silk thread emitted from the silk duct on the ligula; the lining when finished forms a compact and strong cell, the silk being very closely criscrossed backwards and forwards. The following process of the completion of the cross walls at either end of the cocoon, the anterior and posterior sieve membranes respectively, has not been observed. These are constructed with silk to which are frequently attached sand grains and wood shavings; in the centre of each end wall is an opening which is variously shaped (Fig.21 ) usually, however, in the form of a trefoil. Through the posterior opening the anal processes of the terminal segment of the pupal abdomen protrude through the anterior end the bristles of the labrum. The undulating movement of the pupal abdomen causes the anal processes and the bristles of the labrum to work continually in and out of the opening. Thienemann (1905) in Davis (1934) suggests that this mechanism helps to keep the openings free from anything that might block them up and so prevent the flow of water through the cocoon which, as a source of oxygen, is essential to the life of the pupa.

The larva now undergoes a quiescent stage called the prepupa, in which the legs are directed forward, the tibia and tarsus of the metalegs lying dorsal to the head which is tucked in between the legs. Finally after some profound changes the pharate pupal stage is reached, the exuvium is cast, and the movement of the abdomen takes the separated parts to the end of the cocoon where they are frequently found. From here the separate sclerites are finally emitted. The pupal stage proper is now assumed.

iii. <u>The Pupa.</u>

The general morphology of the pupal stage of some British Trichoptera

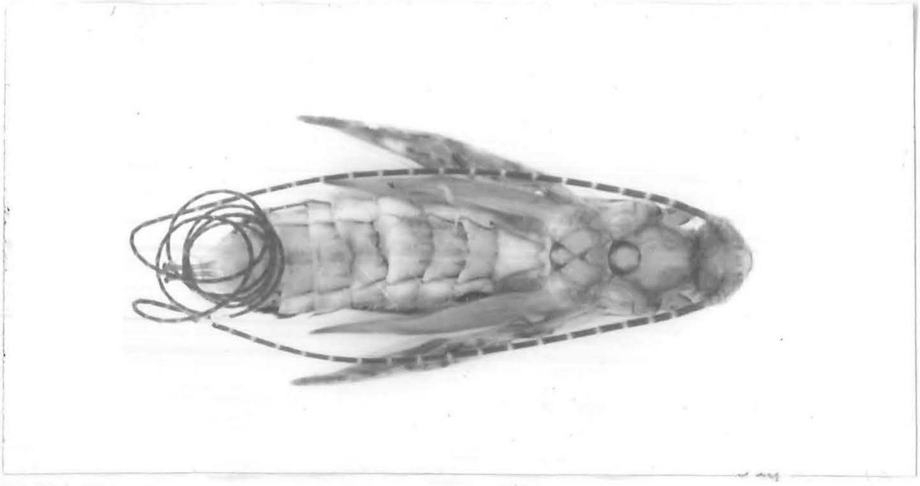


Fig.22a Dorsal view of pharate imago.

5 mm.

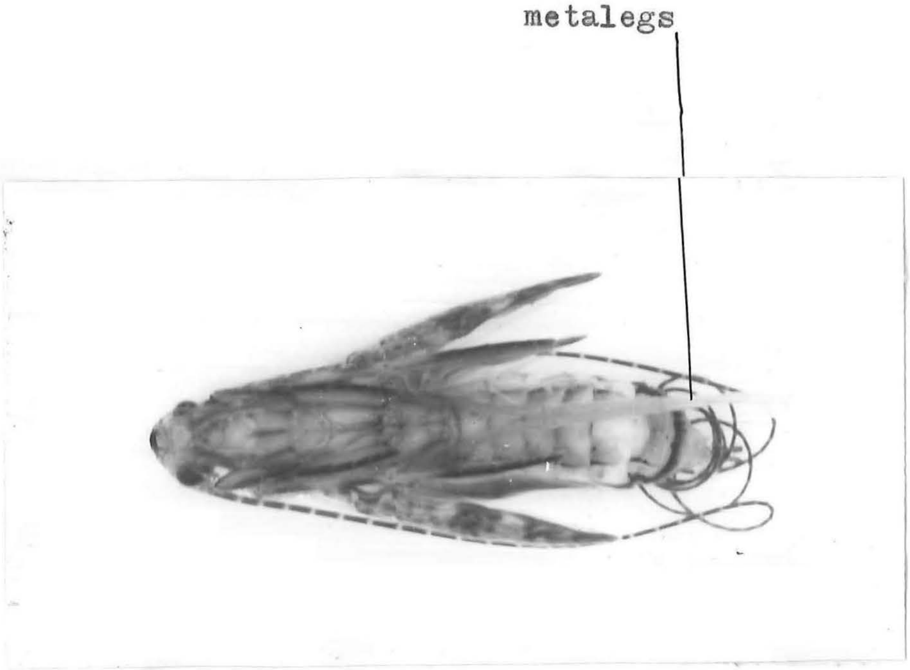



Fig.22b Ventral view of pharate imago.

5 mm.

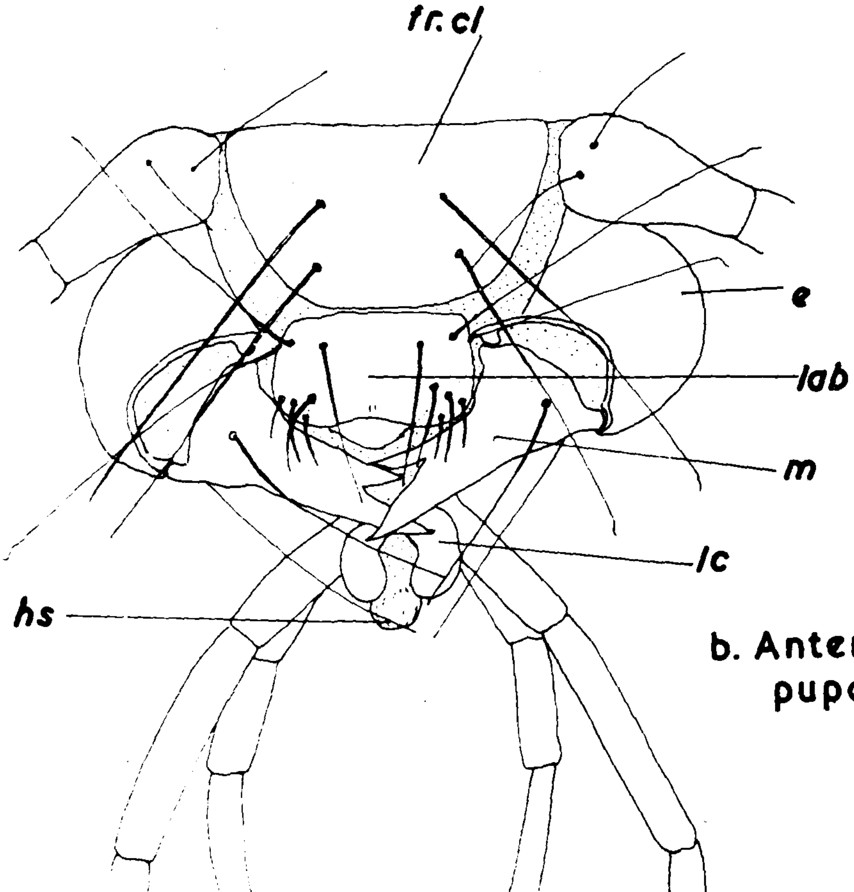
has been described by Hickin (1949), and as this is the most recent general work on the pupal stage, it has been referred to more especially for terminology.

The pupa of T. obsoleta is at first pale in colour, the abdomen is an ochreous-yellow; later in the pharate imago the dark patches on the wings and colouration of the thoracic and abdominal segments and darkened antennae show throught the pupal skin.

Hinton (1946) proposes the name pupa dectica as opposed to pupa adectica, for pupae with mandibles that have a basal articulation and are capable of being moved by the imaginal muscles. This is the position in this species as in all other Trichoptera and therefore the pupa may be called decticous. The antennae, wings and legs lie free in the pupal cocoon.

The general form of the pupa simulates that of the adult insect, which in the pharate imago lies loosely beneath the pupal integument (Fig.22 a and b). The mandibles (Fig.23 a m) project forward from the anterior margin of the fronto-clypeus. They are strongly chitinised with a red-brown tinge. The blade of the mandible is divided to form two large teeth, and the inner edge of the apical tooth is finely serrated. The broad base on its outer edge articulates with the head by means of a well developed condyle, and on the inner dorsal margin of the mandible by a smooth facet (Fig.23a f. )into which fits a projection of the sclerotised ring surrounding the mouthparts. Two large bristles on the outer surface of the mandible are characteristic. When the pharate imago is disturbed the mandibles open and shut; when shut the large teeth cross over in the


a. Pupal mandibles.



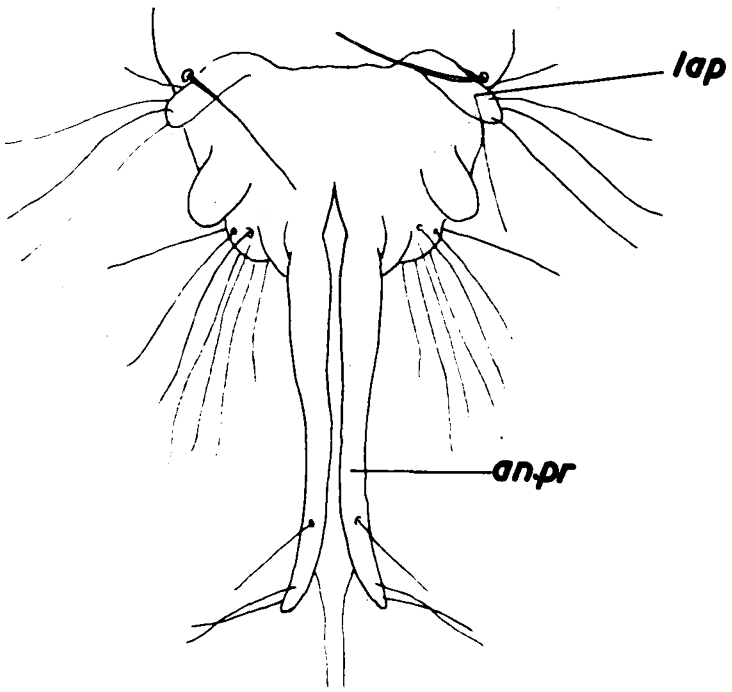

b. Anterior view of pupal head.

Fig.23.

midline (Fig.23 b ). As observed by Hinton (1946 and 1958) the pupal mandibles are in fact articulated by the imaginal muscles, the pupal apodemes being inserted tightly into the imaginal apodemes. These imaginal muscles are only functional in the pharate imaginal stage and after ecdysis the abductor and adductor muscles degenerate, as the mandibles in the adult are non-functional. It is generally suggested that the pupal mandibles are the means whereby the pharate imago makes its way out of the pupal cocoon, by cutting through the sieve membrane. One cocoon was found, however, in which a hole had been cut throught the wall of the cocoon and the sieve membranes were intact. This method has not been observed in this species.

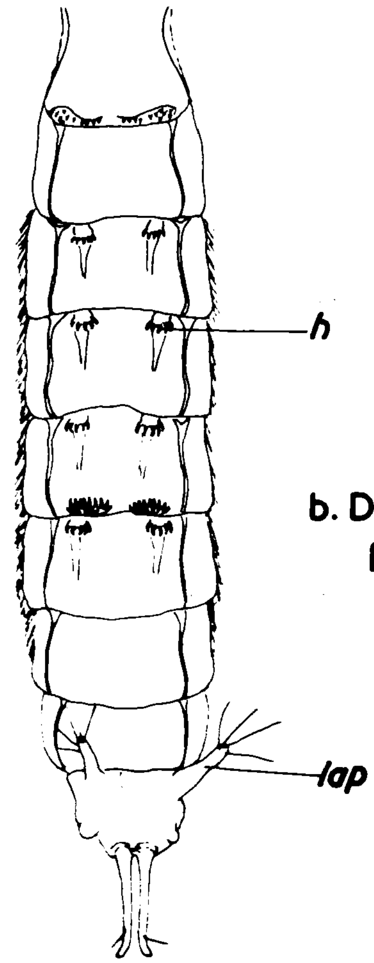
The pupal labrum (Fig.23 b lab.) lies flat against the mouth parts in the same plane as the fronto-clypeus (Fig.23 b fr.cl.). Directed forward from the upper surface are very long black bristles which are said to have a cleansing action on the anterior sieve membrane; long bristles are also present on the fronto-clypeus, probably having a similar function.

Beneath the mandibles lie the maxillary palps which are 5 segmented and the labial palps are 3 segmented. Lying at the bases of the maxillary palps are the lacinia and in the median line is the haustellum (Fig.23 b hs.). The eyes are prominent, pale at first but in the pharate imago they are black. The antennae are from 2½ to 3 times the length of the body. The basal segment is directed posteriorly and from it the antennae lie along the dorsal surface of the eye, close to the anterior wing and finally coil around the posterior abdominal segments (Fig.22 a and b). They are held in position by the forwardly directed dorsal lapettes (Fig.24ad.lap.).




a. Dorsal view of segments VIII and IX.

Fig.24.




b. Dorsal view of pupal abdomen.

The wings are held closely pressed to the sides of the abdomen, the anterior wings reaching as far back as the 6th segment, and both pairs are much smaller than those of the adult.

The legs are free, the coxae of all the legs are closely adherent to the thoracic segments, the femora are directed forward and also are closely pressed to the body, while the tibia and tarsus are directed posteriorly, these segments of the pro and meso legs being free (Fig.25 ) and the tarsi having on the mesolegs two well developed rows of swimming hairs (Fig.25 sh). The tibia and tarsi of the meta legs lie together and along the midline of the abdomen to the last segment (Fig.22 b metalegs). The segments of the integument correspond with those of the adult and the tarsal segment is lengthened to accomodate the tarsal claws.

The abdomen, which is composed of 9 segments, is similar in form to the adult except that the segments are not at all telescoped. The pupal abdomen has a number of special features exclusive to the pupal phase. Along the dorsal surface of segments 3 to 6 are a series of hook bearing processes (Fig.24 bh.pr), the hooks being directed posteriorly. The posterior margin of the 1st segment is modified in the form of two rows of teeth (Fig.24 b ); it is from this process that the undulating movements of the abdomen pivot, the teeth forming an anchor in the silk lining of the cocoon. That this is so is supported by the fact that they are absent in the forms that do not undergo undulating movements of the abdomen in the pupal stage (Hickins 1946). The posterior margin of the 5th segment has a pair of hook bearing plates of a slightly different nature to the others and the hooks are directed anteriorly. Hickin (1946) says of these hook bearing plates

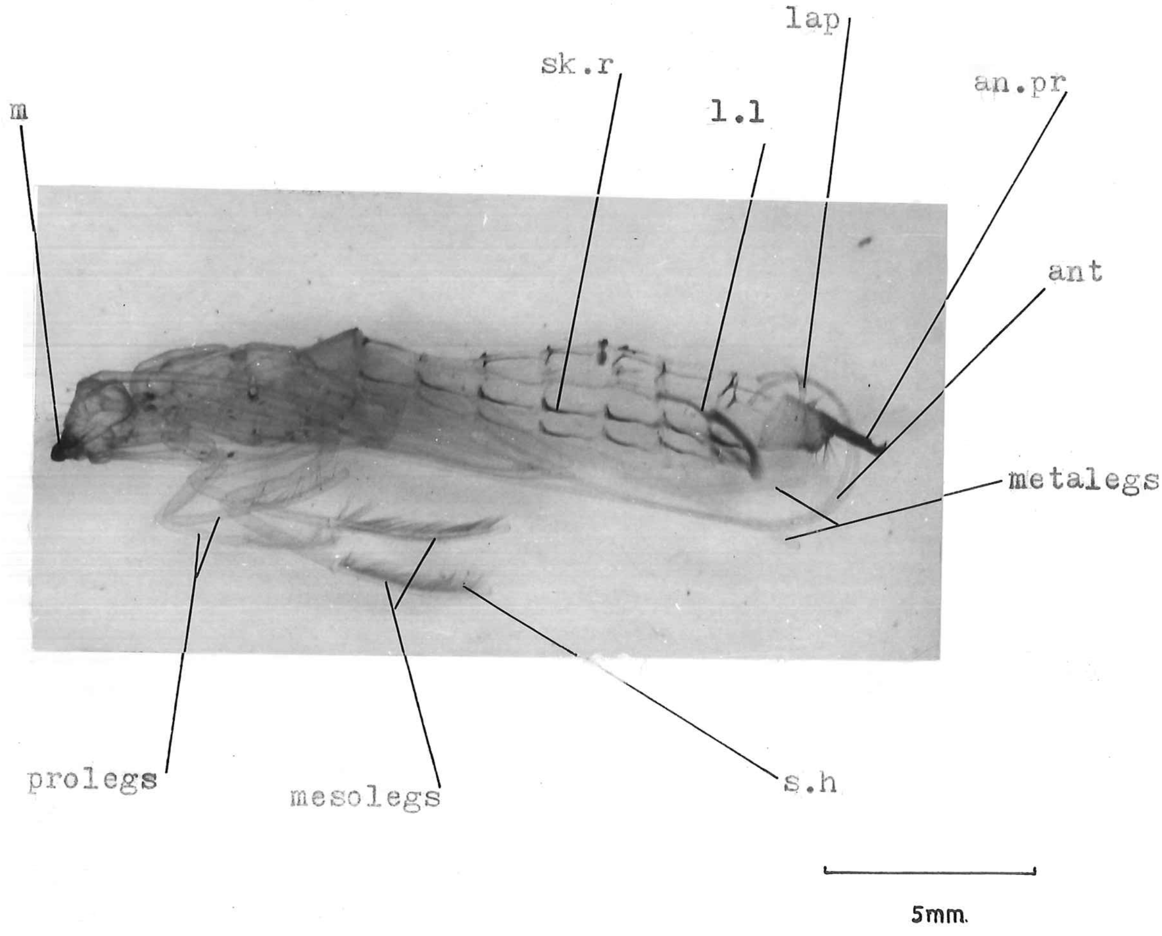



Fig.25 Pupal exuvium.

that they are of a locomotory function. The purpose of the dorsal lappets as already indicated is to hold the coiled antennae in position.

Posteriorly segment 9 ends in two long slender anal processes (Fig.24 a an.pr.) with specialised setae, these processes normally move in and out through the posterior sieve membrane maintaining a clear orifice. The imaginal genitalia can be seen beneath the integument of the 9th segment.

A lateral line (Fig.25 l.l.) is present from segment 3 to segment 8 where the lateral line passes down ventrally and almost meets in the mid line. Compared with the larval lateral line the hairs are much longer. In the exuvium (Fig.25 sk.r) 4 pairs of skeletal rods may be seen in the abdomen from segment 2 to segment 8.

Gills of the same form as those of the larva are present from segment 2 to segment 7.

iv. Duration of the Pupal Stage.

This particular aspect of the pupal phase has been calculated only from field observations. The first sign of the onset of pupation was noted during the fifth week in August, and it was not until the first week in October, approximately five weeks later, that the first imagos of the season were caught. The time lag between the beginning of pupation and the first signs of activity of the imago indicate that the total length of time taken for pupation is approximately 5 weeks.

The site commonly chosen for pupation makes ready observation of the individual specimens difficult. Few isolated observations have been made in order that some idea of the time taken in the prepupal, pupal and pharate imaginal stages might be gained. One of these observations has given some indication of the time taken for a prepupa to reach the pharate pupal stage. A 6th stage larva was found boring into a piece of solid wood, this process taking 1 day; the tunnel was then lined with silk and the pupal cocoon finished by the end of the 7th day. The prepupal quiescent stage, then, lasted 10 days by which time the pharate pupal stage was reached, the ecdysis having not yet occurred. Several examples of 6th instar larvae burrowing into solid wood prior to pupation have been noted and every time the old case has been freed by the larva cutting through the silk threads fastening the case to the entrance of the new tunnel.

Other observations of time taken in the phases of pupation have been too isolated, and no accurate times have been obtained. In conclusion it may be said that time taken for pupation is approximately 5 weeks. Hudson (1904) states 3 - 4 weeks, so the duration of time for the pupal stage should be rechecked.

iv. <u>Pharate Imago and Emergence.</u>

The pharate imago, as Hinton points out, is actually the first imaginal stage still retained within the pupal integument, and as compared with the pupa is an active stage. As in the adult the pharate imago is clothed with black and white hairs which give colour to the pale skin; the abdomen is coloured in the pattern characteristic for the adult. The only point of contact with the pupal integument is that of the pupal apodemes of the mandibles lying within those of the pharate imago, the movement of the mandibles not therefore being a pupal characteristic, but an imaginal one.

After having worked its way free of the cocoon the pharate imago swims by means of its pro. and mesolegs to the surface, the meso-legs being the most active during this process, which is aided considerably by the hairs on the tarsi of the pupal integument of pro. and mesolegs. The pharate imago then swims along the surface in jerky movements, the action somewhat resembling that of a 'backswimmer' (Notonectidae). After a few minutes a split occurs along the posterior head region, the thorax and a little down the abdomen. The head, pro. and mesolegs, antennae, wings, metalegs and finally the abdomen are pulled free of the exuvium. Almost instantaneously the imago flies from the pupal skin and flutters in its characteristic zig zag flight above the water, and comes to rest on a blade of grass or some other type of vegetation along the stream edge. The teneral imago is very pale and does not assume its normal colouration for several hours.

Time of emergence coincides with the time of maximum flight activity, which is at dusk, but does occur for 1 to 2 hours later. Emergence has not been observed after this time. Specimens seen emerging in the laboratory have done so at a time which in most cases corresponds to that observed in the field. Scott (1936) has done some work on the factors governing the hour of emergence of adults from their pupae, and for his experiments he used a Phycitid moth. From his results he comes to the conclusion that emergence rhythm is inherited, and a 24hr periodicity seems to be a basic part of the emergence rhythm. The time of maximum emergence can however be changed by altering for example, the time of maximum temperature.

It is probable that in the case of these dusk flying Trichoptera which live in streams with a moderately stable temperature, it is a fall in light intensity which induces the hour of maximum emergence. This is only conjectural, and is of course subject to some future experimental work. It can safely be said, however, that the maximum emergence time corresponds closely to the main flying time of the imagos, and that actual emergence takes place from the surface of the water; this, as stated by Siltala (1906 d) in Davis (1934) occurs in some other families of Trichoptera including certain Leptoceridae.

## 1/3 The Imago

### i. Introduction.

The taxonomic description of the imago of Triplectides obsoleta has been given by Mosely and Kimmins (1953) who use the description given by McLachlan (1862) of a female, for the species identification, and Mosely and Kimmins then continue with a description of the male genitalia. In this thesis under the subsections dealing with the female and male imago, these above descriptions are quoted. They are added to where necessary, and are followed by some comparative morphology of the male and female imago. In a few instances some differences in detailed morphology, of the male genitalia in particular, will be given. Aspects concerning the biology of the imago are dealt with in succeeding subsections.

### ii. Material and Methods.

The adults were obtained without undue damage, when caught by the methods outlined in the general introduction. A small insectory measuring 2'4" x 1' x 2' was made; adults were freed into this, and kept alive quite successfully. On the floor of the cage were clumps of long grass, and the 'flies' were supplied with water to drink. When specimens were found copulating or showing signs of pairing, they were isolated in smaller cages. At this juncture it was still thought that the female of this species was viviparous. Accordingly it was thought that if in fact females that had been fertilized could be kept alive as long as possible, some idea of the stages in development of the egg through to the young 1st instar would be obtained. A freshly emerged female was placed under a bell jar with three males, but no copulation was observed.

Material to be used for exoskeletal examination was boiled in a 10% Potassium Hydroxide solution, neutralized in Glacial Acetic Acid, transferred to water and then 70% alcohol. Male and female genitalia were dissected out and mounted separately. Whole mounts were also made of the heads of males and females, with mouth parts intact; mouth parts were also mounted separately. Whole mounts were made of the antennae and wings of male and female imagos. No special techniques were employed in the preparation of wings prior to mounting, as the venation could be seen without denuding them.

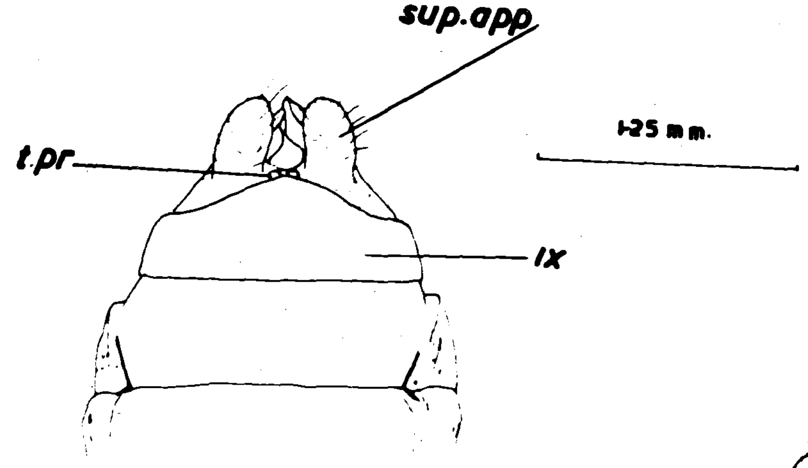
iii. Description of the female.

McLachlan bases his description of the species on a description of a female which is a type specimen in the Hope Department, Oxford University. The description reads as follows: "Antennae fuscous, narrowly annulated

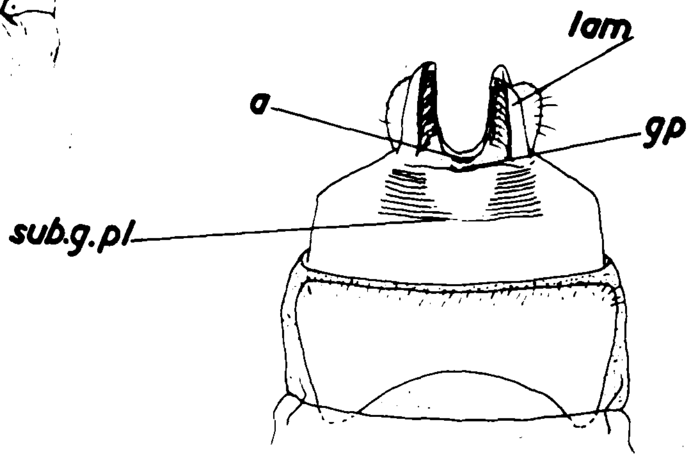
> with pale grey; palpi greyish fuscous, head and thorax testaceous, the metathorax paler; abdomen greyish brown; legs grey, tibiae and tarsi blackish; anterior wings very pale grey clouded and irrorated with dark grey; the most conspicuous markings are an oblique, almost obsolete fascia before the middle, and an indistinct blotch at the pterostigma, posterior wings semi-opaque, whitish, slightly irridescent.
>
> Long. corp. 6 lin.; exp. alar. 17 lin."

To this Mosely and Kimmins add that the spur formula is 2,2,4.

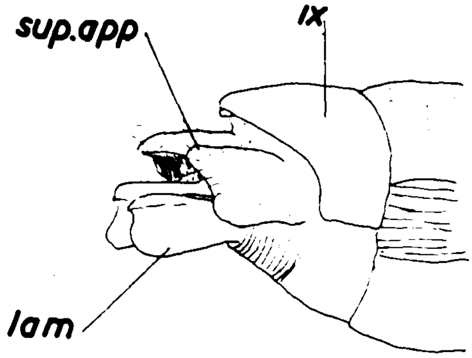
The abdomen is more of an olive green to brown in colour when the adult is alive, losing colour soon after death. The abdomen is considerably larger than in the male due to the development of the colleterial glands and the ovary, in the more mature specimens. The abdomen ranges from 6



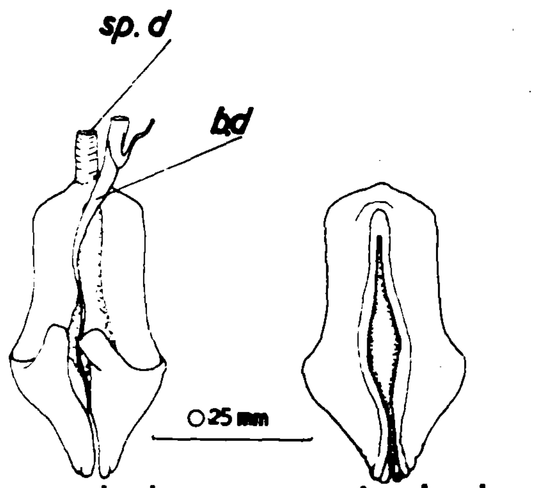

a. Dorsal view




b. Ventral view




c. Lateral view



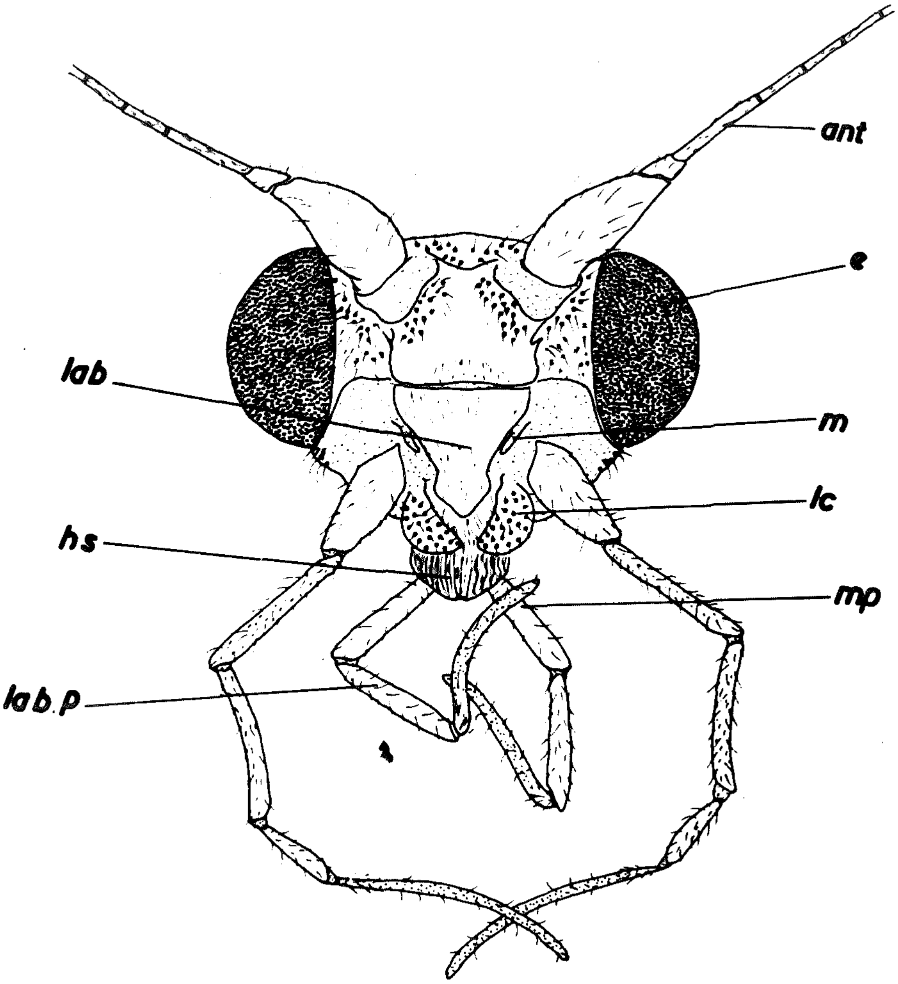

dorsal view ventral view

d. Vaginal structure

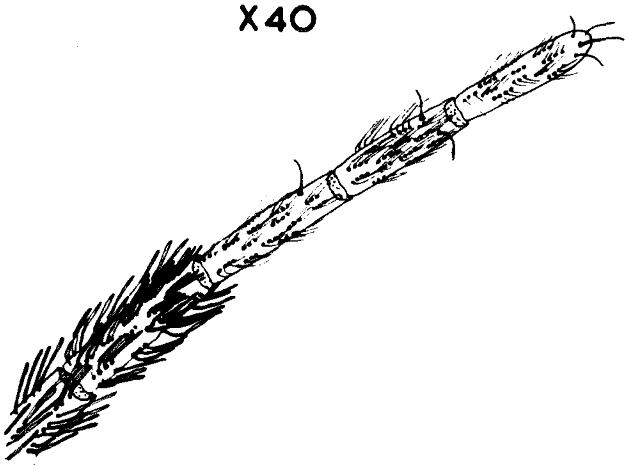
Fig.26. Terminal segments of female and vaginal structure.

to 10mm in length, and from 2.5 to 3mm in width. The shape of the abdomen is often more rounded than that of the male, but as in the male the abdomen narrows down to a thin waist where it meets the metathorax. The pleural areas of the abdominal segments are pale and membranous, the first seven pleurites bearing spiracles. Intersegmentally are borne white filamentous structures which Betten (1934) suggests may be regarded as persisting tracheal gills. The first seven segments are normally developed. On the terga and sterna are chitinous ridges which help to preserve the shape of the body. Segment 8 is slightly narrower than 7 and ventrally is chitinised to form a sternal plate. The tergum of 9 is triangular in form (Fig.26 a ix) and is smaller than the flat ascending subgenital plate of the sternum (Fig.26 b sub.g.pl.). The posterior margin of this plate is produced into two chitinous lamellae projecting well beyond the posterior end of the abdomen (Fig.26 b lam.). Ventrally situated at the base of these lamellae is the genital opening (Fig.26 b and c; gp.), and dorsally to this is the anal opening (Fig.26 b and c; a.) still within segment 9. Just beneath the apex of tergum 9 are two small truncate protruberances (Fig.26 a tpr.). Segment 10 is smaller than the preceding segments (Fig.26 b; x) and is only lightly chitinised: it bears two superior appendages, which are rounded in shape as seen from above (Fig.26 a ), but pointed in lateral view (Fig.26 c; sup.app.). The inner surface of these appendages is concave and smooth, while the outer edge is convex, fleshy and covered with numerous short hairs.

In the cleared preparations the chitinous vaginal structure (Fig.26 d v.) is easily seen. In one specimen, stained in aniline blue according to




a. Anterior view of adult female head.
X40



b. distal segments of antenna. x100

c. distal segment of maxillary palp.

x1500

Fig. 27.

methods outlined in 1/1, the vaginal structure shows up especially well. This structure will be dealt with when the anatomy of the reproductive system is described.

The head (Fig.27 a) is wider than it is long and wider than the thorax. The eyes are black and very prominent. The antennae, while at least twice the length of the anterior wing, and four times the length of the body, have an average length of 4.0cm. The antennae are annulated with dark rings at the proximal and distal end of each segment. They are clothed with flattened hairs. The basal segment is bulbous, the second is smaller, and succeeding segments are longer than wide. The last three segments appear white, since they are only clad with very fine white hairs (Fig. 27b). It would appear from observations made, that these long antennae with the nearly naked last few segments are very efficient sensory structures, which seem to be especially active when pairing.

The mouth parts are similar for both male and female and will be briefly described here. The labrum (Fig.27 a lab) arises just ventral to the frontoclypeus, laterally it narrows to a waist then bulges slightly and ends in a point. It usually lies flat. The mandibles (Fig.27 a, m. ) are small flaps of skin at either edge of the base of the labrum and as a rule lie beneath it, directed towards the mid line. They do not meet in the centre and are therefore non-functional. The maxilla consists of cardo, stipes and a 5 segmented palp at the base of which is a well developed lacinia. Commonly this lobe has been called galea, but Crichton (1957) on evidence of musculature in adults which he has examined maintains that this lobe has a cranial flexor muscle inserted on it, and should therefore be

called lacinia. Crichton points out that Imms states (1944): "It is noteworthy that no cranial flexor or its counterpart is developed in connection with the insectan galea and Das (1937) in his investigation of the mouth parts of insect larvae, described a cranial flexor to the single maxillary lobe of the larva of Anabolia (Limnephilidae)".
He (Crichton) therefore interpreted this lobe as lacinia. The segments of the maxillary palp vary slightly in length. The first segment is half the length of the second and wider. The second and third segments are the same length, the fourth segment is half the length of the third. The fifth segment equals the total length of three and four combined.

Situated beneath the labrum in the mid line is the haustellum (Fig.27a hs.), the cuticle of which is soft and flexible. When fixed this organ is frequently retracted, so that to all outward appearances it is lacking. Slight pressure on the thorax, however, while fixing will ensure that the haustellum is protruded. The cuticle of the anterior portion of the haustellum is produced into longitudinal ridges so as to form many channels, the endocuticle of which is transversely ribbed. The exocuticle from each transverse rib is drawn out into a compound pectinate hair. The detail of these cuticular structures is described by Crichton and the main features are the same as those that have been observed in this species. Dispersed in the channels of the apical region of the haustellum are numerous sensillae which according to Crichton are supplied with nerve endings.

The labial palps are three segmented (Fig.27a,lab.p.) and arise from a fused base beneath the haustellum. Concerning the labium Crichton says: "The unsclerotised posterior surface of the proboscis, between the

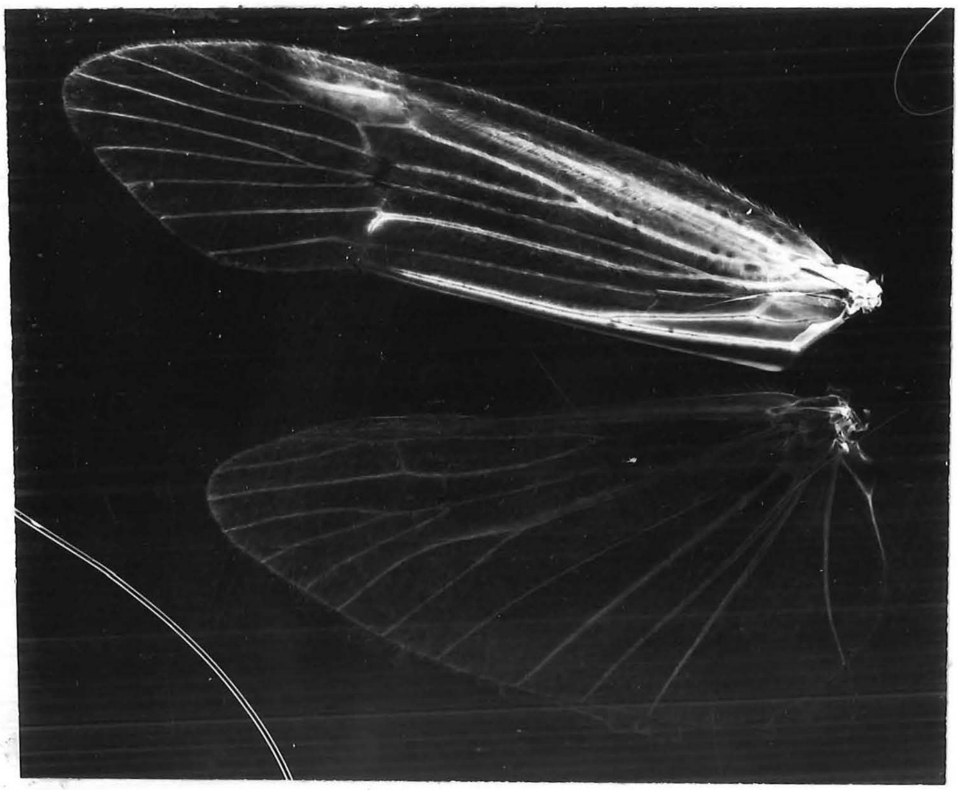


Fig.28 Female wings.

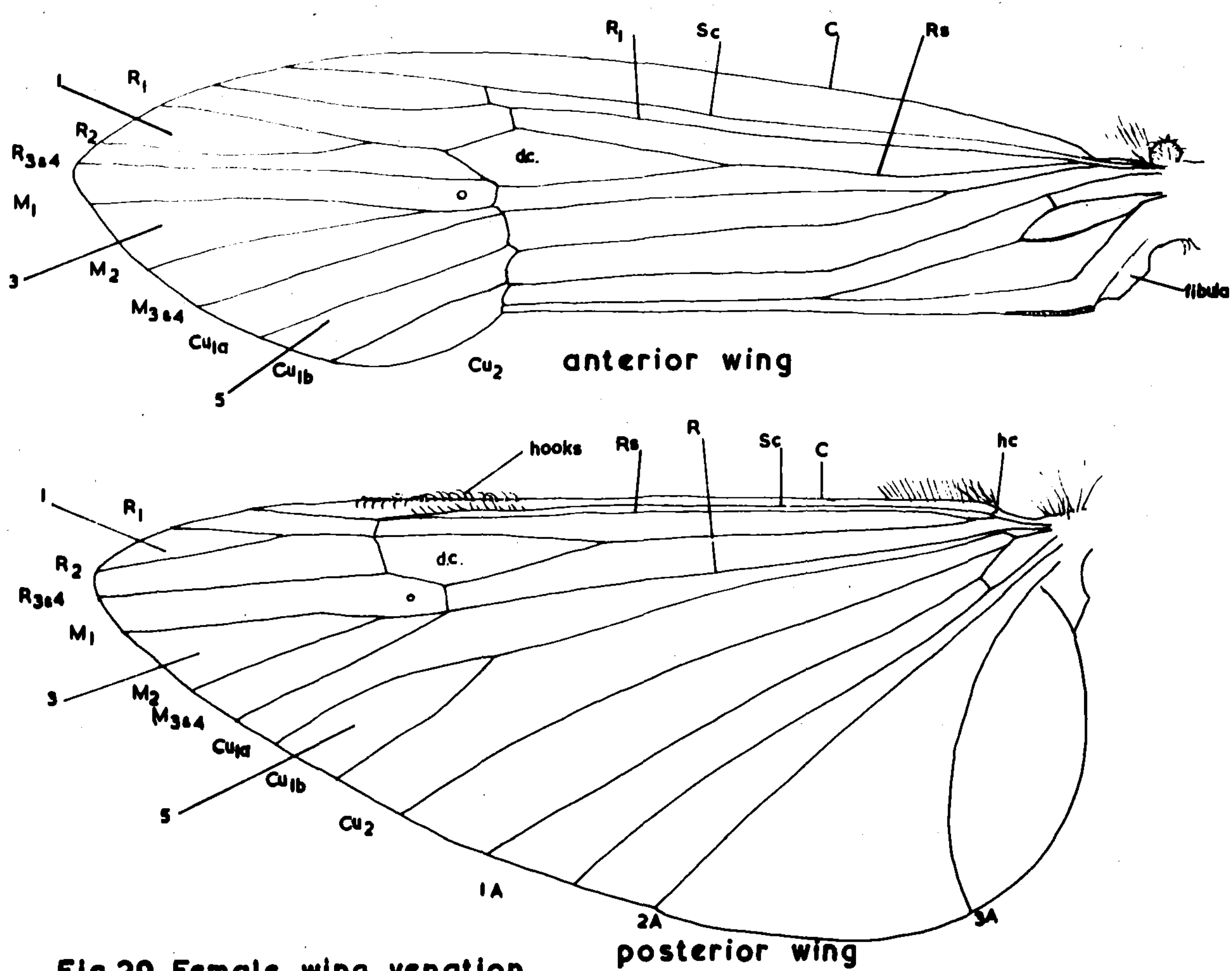



Fig.29. Female wing venation.

laterally placed cardines and stipitites of the maxillae is presumably derived from the labium. This surface is continued into a terminal region, which projects a little way beyond the base of the haustellum, and bears the well developed labial palp. Between the bases of these palps there is a deep cleft, but there are no lobes representing a ligula."

The head and thorax are clothed with black and white hairs. The maxillary palp is covered with hairs of two types, one, the same as those on the body and two, flattened hairs; (Fig.27 c) minute spines are dispersed between them. The last segment of the labial palps and maxillary palps have a number of sensory pits (Crichton).

The wingbearing segments of the thorax are well developed, and are similar in form to those of the pharate imago (Fig.22 a). The prothorax is hardly perceptible and is only seen in lateral view. The wings (Fig.28) are slightly larger than in the male (Fig.30) and have $M_1$ and $M_2$ divided to give apical cell 3 (Fig.29.). A comparison of wing measurements is given in the following table:

| | Female | Male |
|---|---|---|
| Length of anterior wing | 16mm | 15mm |
| Width of anterior wing at widest part | 4.5mm | 3.5mm |
| Length of posterior wing | 13.5mm | 11mm |
| Width of posterior wing at widest part | 6mm | 5mm |
| Wing span | 36mm | 33mm |

Table 6. Measurements of Male and Female Wings.

It should be pointed out here that the wings drawn in the key for the species by Mosely and Kimmins show the cross vein closing the discoidal cell as being concave. An examination of the females, however, shows that the typical cross vein closing the discoidal cell in the anterior wing differs from the male (Fig.31 d.c.) in that it is usually straight (Fig.28 d.c. and Fig.29). Another point of difference is that the downward production of the angle of the discoidal cell, which may be taken as the most important character separating Triplectides from other genera in the subfamily Triplectidinae, is fairly well marked in the male but only very slightly developed in the female, if at all (cf. Fig.28 and Fig.30). On the outer edge of the costa and radius are two rows of well developed hooks (Fig.29). They are curved inwards towards each other and catch on to the compound anal vein of the fore wing, which here is partly fused to the hind margin of the wing for some distance. The fibula as in many Trichoptera is about half as broad as it is long, and as Betten points out, it is broadly joined to the wing so that there is no possibility of its passing under the hind wing. Instead, it reaches over the latter for some distance, and by the development of hairs on the under surface holds on to the bases of cubitus and the anal veins. The fibula has a strong tendency to fold under the wing; this serves to keep it closely applied to the hind wing during flight. Hairs are well developed along the costal margin of the hind wing, especially on the humeral angle. These mechanisms joining wings during flight are present in both male and female adults.

The legs are developed for running as in other Trichoptera and there is no need for redescription. The spurs formula is 2,2,4, for both male and female.

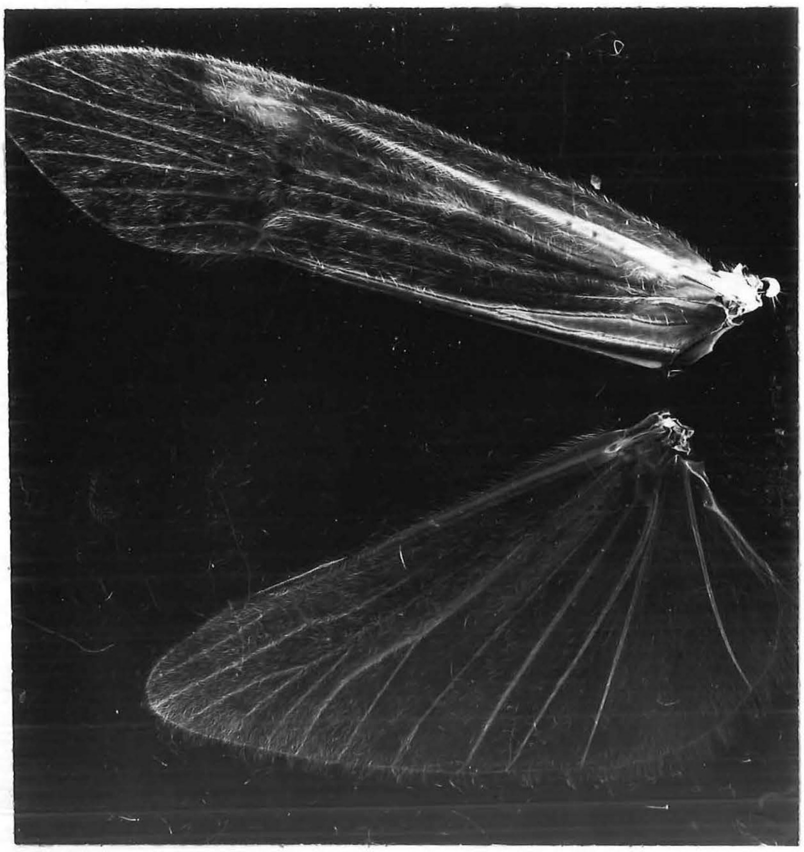


Fig.30 Male wings.

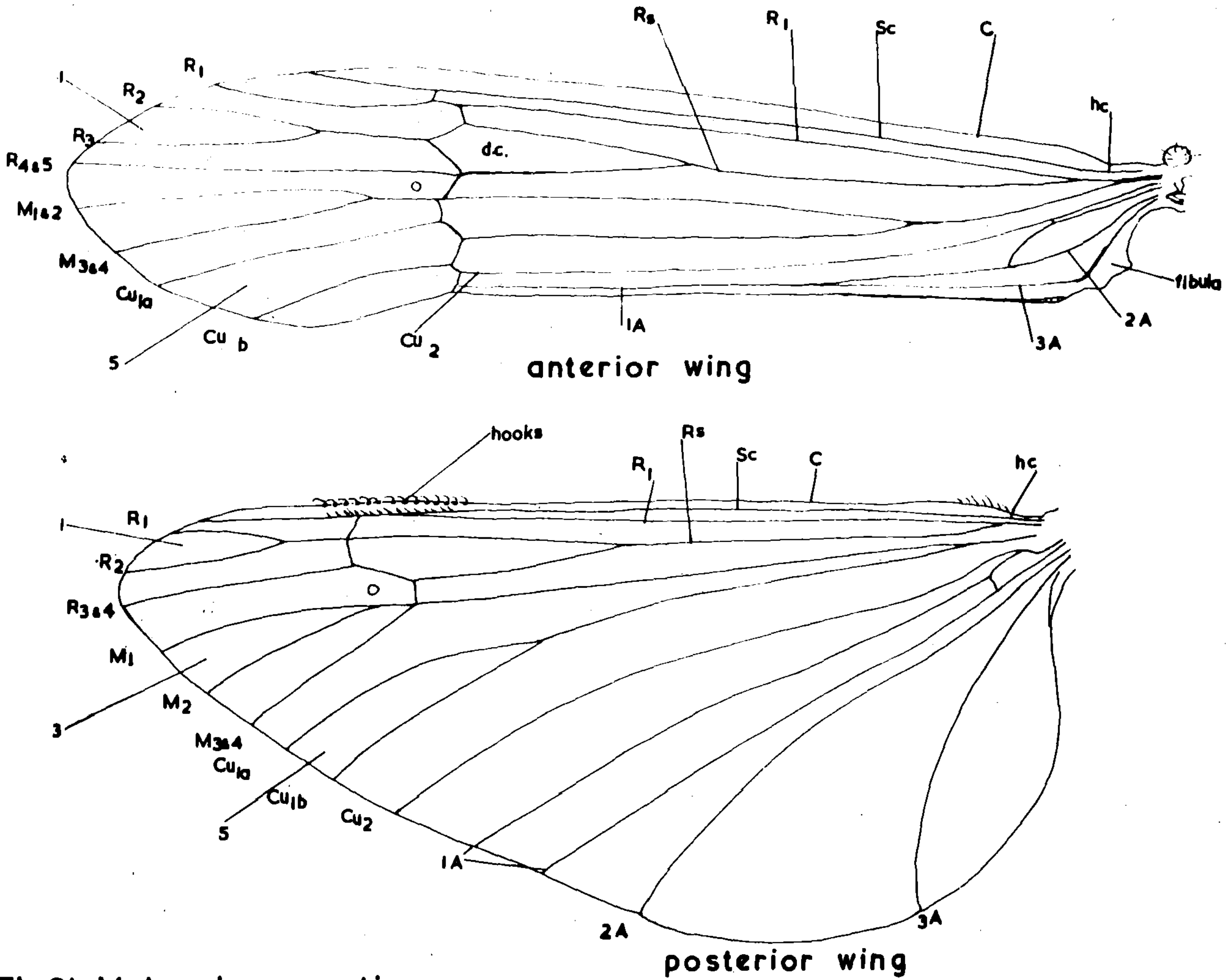



Fig.31. Male wing venation

iv. Description of the Male.

The head is slightly broader than that of the female, but is otherwise similar in form. The antennae are usually longer than in the female, sometimes measuring as much as 4.5 cm. The thorax has the same form as that of the female. A comparison of the male and female wings is given above and the male wings are figured in Fig.30 and Fig.31.

The abdomen of the male is slender and tapers towards the terminal segments. The terga and sterna are a dull green brown in colour, while the pleura are pale and membranous. Segments 1 to 8 are normal and the remaining ones are modified to form the external genitalia.

To begin with, Mosely and Kimmins (1953) description of the male genitalia will be given. Since 1953 papers by Nielsen especially 1957 and other works which will be referred to later have helped considerably in understanding the anatomical detail of the genital segments and their appendages in the male Trichoptera. Also the number of terms used for the different parts of the copulatory apparatus has led to much confusion; the relative merits of the use of some of these terms will be discussed. Finally the genitalia in specimens collected from the type locality show some variation in form.

The taxonomic description given by Mosely and Kimmins is as follows: "From above, distal margin of the 9th segment produced into a triangular plate with an acute apex; there are two small processes set close together under the apex of this plate, but only visible in a balsam preparation. Superior appendages arise from beneath the dorsal plate, rather stout and long, slightly longer than the upper penis cover which, from above, has a truncate apex and, from the side, a rounded excision on its upper margin towards the base. Penis slender at the base, broadening gradually to a cup-like apex in which are set two rather broad nodules with two black upwardly pointing hooks between them. from beneath, there is a Y shaped ridge extending across and slightly behind the lower apical margin. Inferior appendages three branched: the first branch is deeply angulated along its inner margin at a point rather more than midway towards the apex; in the angle is set a short and rather wide second branch, with strongly chitinised bifurcate, claw shaped apex; the third branch arises from the lower basal margin of the appendage, as seen from beneath; it is short, fringed, and extends to the base of the second branch. The inner plates arise from the inner margins of the appendages towards the base and are sinuous, curving towards the penis. Margin of the ninth ventral segment is produced at its centre in a rather wide, triangular process; ventral margin of the eighth sternite sharply excised at its centre."

Before any discussion can ensue the terms to be used will have to be understood. Snodgrass (1935) gives the term penis to mean one of the paired intromittent organs of certain insects, or the usual phallic organ. The term phallus then is the unpaired penis, or median intromittent organ including the phallobase, the aedeagus, the endophallus and various processes of the phallobase and the aedeagus if present. The term penis is frequently used interchangeably with aedeagus or with phallus; thus it is not homologous throughout the insect class and Tuxen (1956) proposes to avoid the ambiguous term penis by the use of the term phallus or

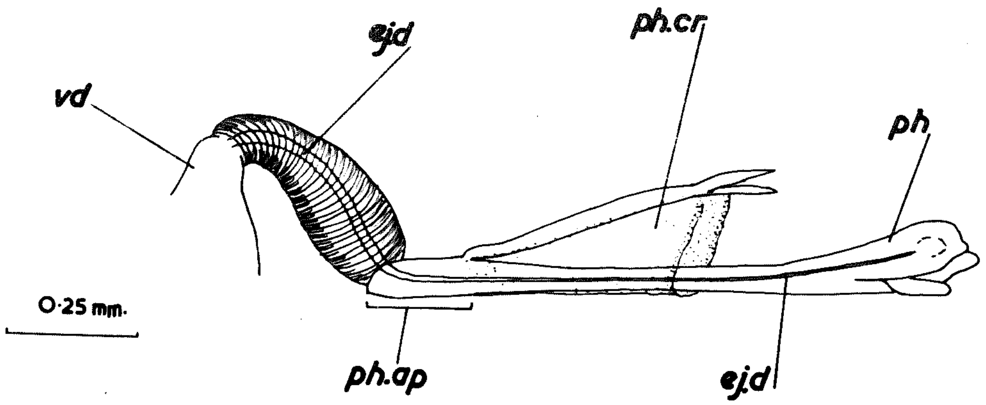



Fig. 32. Lateral view of phallus.

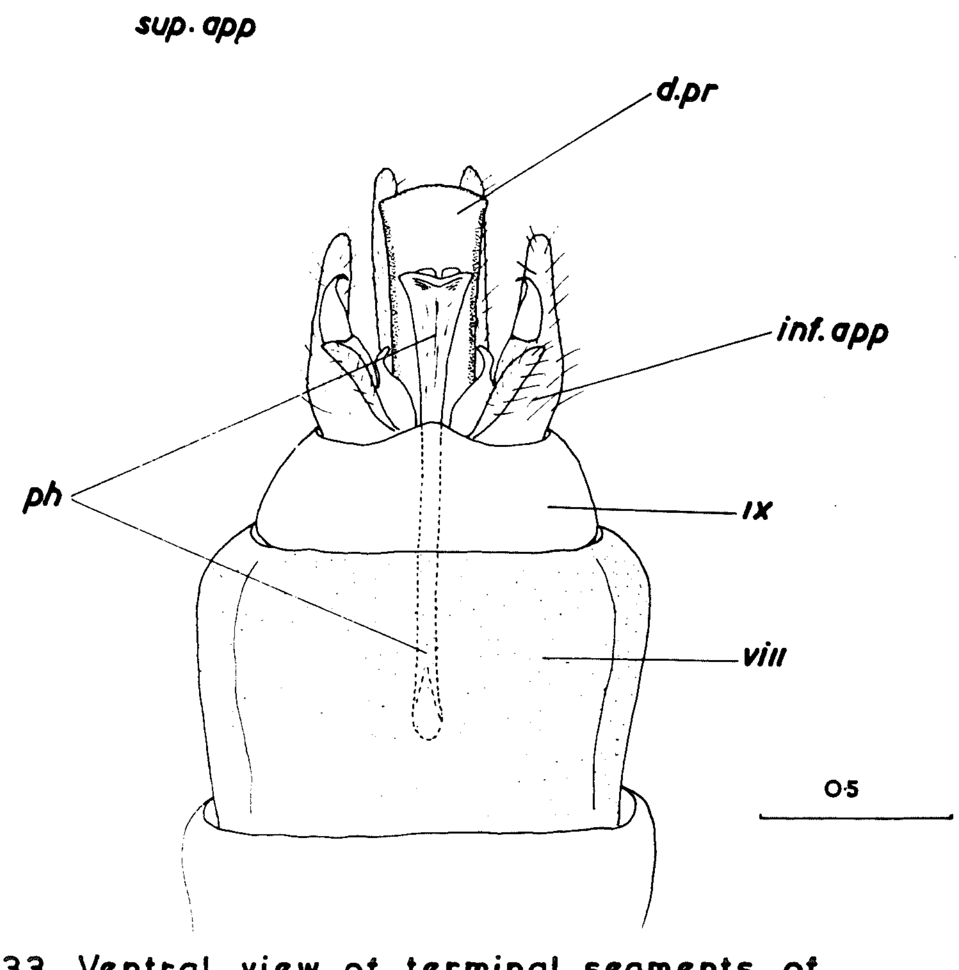
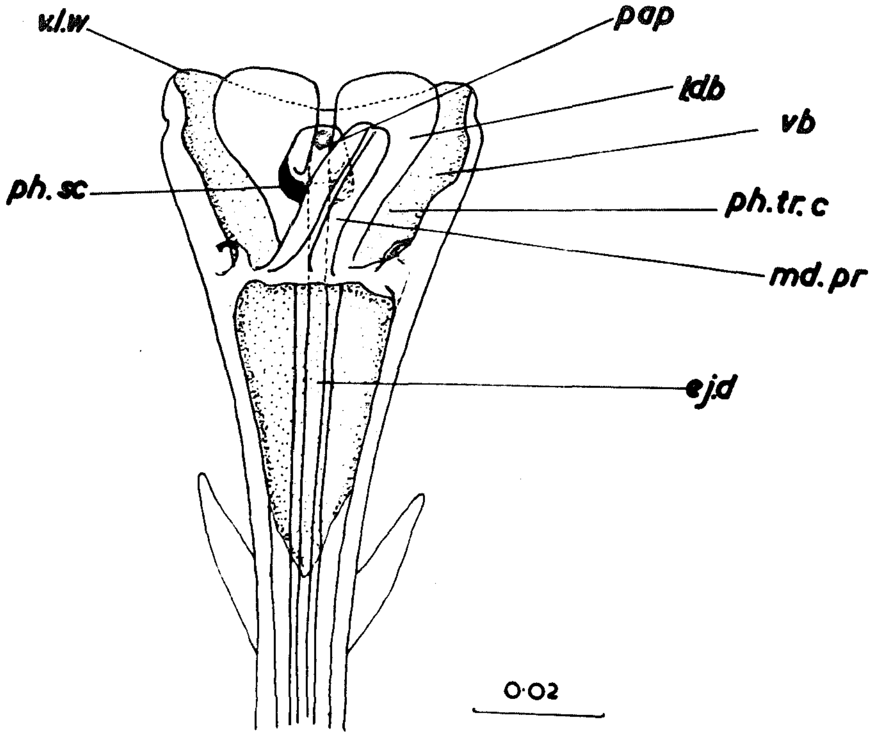



Fig. 33. Ventral view of terminal segments of male to show position of appendages.

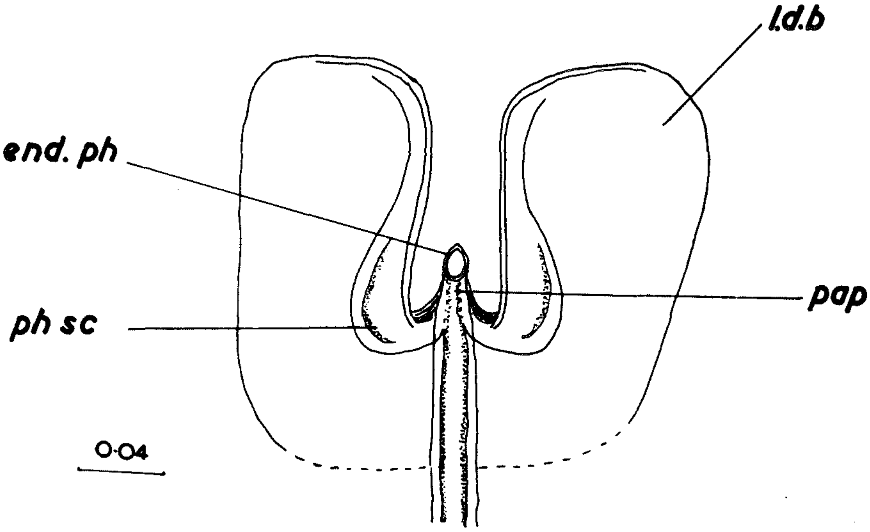
aedeagus respectively. Nielsen (1957) uses the term phallus as designating the whole copulatory organ, whether divided or not. If divided the proximal part is called the phallobase as opposed to the more distal adeagus. In this work Nielsen's terminology will be used.

Concerning segment ten several names are used: dorsal plate, penis cover, and upper penis cover being the most common. Mosely and Kimmins (1953) use the term upper penis cover and the term dorsal plate is used to denote the tergum of segment nine. Tuxen uses the term dorsal plate synonymously with segment ten. It is proposed here that this median, most readily seen part of segment ten should be better termed, in this species, dorsal process (Fig.35 a d.pr.). Superior appendages arise from the anterior margin of segment ten (Nielsen 1957) beneath segment IX and are as long as the dorsal process of this segment. The inferior appendages arise ventrally from segment nine and their description is in accord with that given above by Mosely and Kimmins.

According to Nielsen (1957) the original phallus ended in a dorsal and ventral branch, as seen in the Rhyacophylidae and some other isolated genera. In <u>T. obsoleta</u> the phallus is undivided and ends in what could only be called a dorsal and a ventral branch (Fig.32). The undivided phallus is here represented by the aedeagus. In this case the undivided condition seems to have arisen in the third of the three ways proposed by Nielsen: "This condition may have arisen by reduction of the whole phallobase, a direct continuation being established between the phallic apodeme and the aedeagus" (Fig.32 ph.ap.; ae.). "This process is accompanied by the reduction of the aedeagal retractor." This muscle appears to be absent




a. dorsal view of distal phallus.



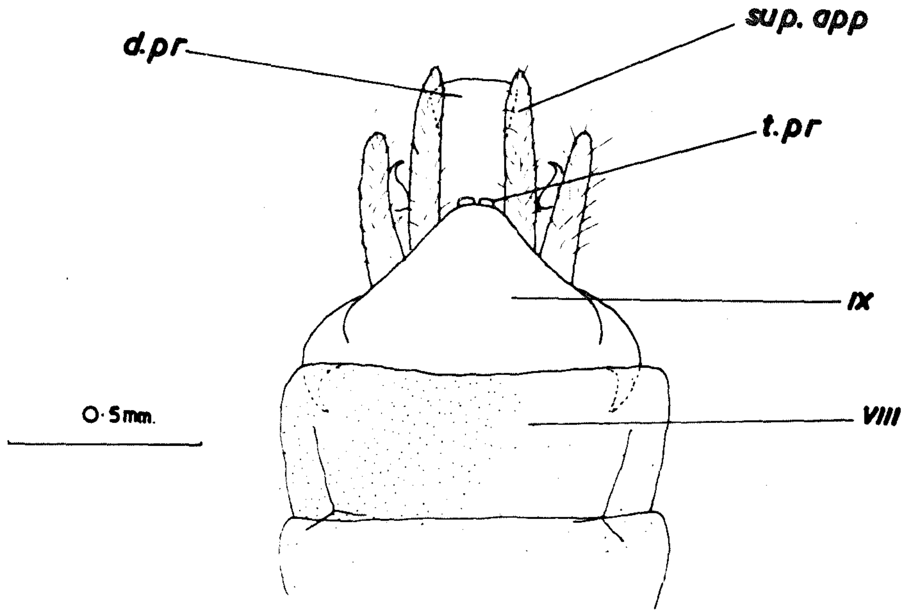

b. dorsal branch of phallus.

Fig.34.

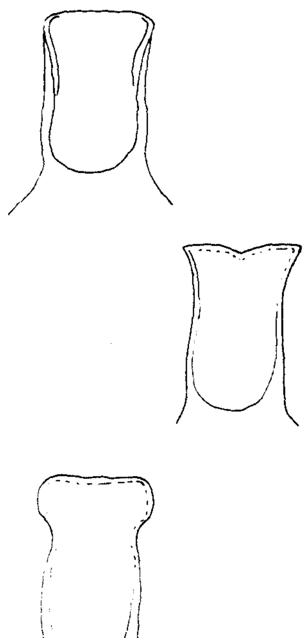
in T. obsoleta. Nielsen says that this condition is seen in Leptoceris, a genus of the same family as Triplectides, but a different subfamily, may have arisen in the same way.

The phallus is a retractile organ, and when withdrawn, at least half its length lies within the abdomen (Fig.33 ph.). The phallus is a long slender structure about 1.0mm in length. Proximally there is a short phallic apodeme (Fig.32 ph.ap.) which receives the ejaculatory duct (Fig. 32 ej.d.).

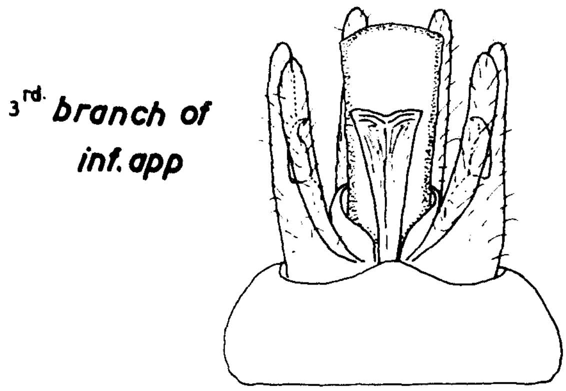
Distal to the phallic apodeme arises the deep phallocrypt in which lies the phallus, (Fig.32 ph.cr. and ph.). The phallocrypt is mainly membranous, ventrally it is sclerotised in the form of a Y, the two arms of the Y together with the rest of the phallocrypt form a tube through which the phallus projects (Fig.32). The distal end of the phallus widens to form a spoon shaped structure and there are two short branches. The ventral branch is sclerotised and extends laterally to give two lateral wings (Fig.34 a v.l.w.). Above this ventral branch is the phallotremal cavity (Fig.34a ph.tr.c.). Dorsally there emerges the second branch, which is divided into two lobes (Fig.34 a l.d.b.). This dorsal branch carrying the distal end of the ejaculatory duct, opens on a 'papilla' between the base of the lobes (Fig.34 a pap.). The endophallus (Fig.34 b end.ph.) is small and forms the end of the papilla. The dorsal branch of the phallus is shown in more detail in Fig.34 b. It is only slightly sclerotised, medianly at the base of the lobes are two sclerites, phallotremal sclerites (Fig.34 b ph.sc.). This dorsal branch of the phallus is distendable. Medianly above the dorsal branch is an unpaired structure




a. dorsal view of typically shaped terminal segments.



b. variation in the form of segment ten.




c. ventral view to show long third branch of inferior appendage.

Fig. 35.

referred to as the median dorsal process (Fig.34 a m.d.pl.). Probably this has some function during copulation.

Mosely and Kimmins (1953) when describing the 'penis' state that it broadens gradually into a cup like apex in which are set two rather broad nodules with two black upwardly pointing hooks between them. Now the two 'rather broad nodules' are presumably the lobes of the dorsal branch, but the two upwardly pointing hooks between them have not been located and it can only be assumed that they may represent the phallotrenal sclerites, although these do not really resemble the hooks that are figured by Mosely and Kimmins (1953).

A dorsal view of the terminal segments is given (Fig.35 a), the shape of segment ten is seen in its more typical form. However, within the species there is some variation in its shape. Frequently the posterior margin is excised (Fig.35 b) and laterally the edges may be straight or waisted slightly, before the posterior end. Ventrally it is always concave and the anus opens beneath at its base. The superior appendages may be rounded or pointed.

The detail of the inferior appendages is seen in Fig.33. In the specimens investigated, however, the third branch may either extend as far as the base of the second branch as described by Mosely and Kimmins or it may be longer; in this case the first branch is also slightly longer than in Fig.33. This feature of lengthening of the first and second branches of the inferior appendages is shown in Fig.35 c. The ventral margin of the eighth sternite shown by Mosely and Kimmins is sharply excised: this feature has not been found in any of the examples seen.

Basically the genitalia is constant in its composition, but there does occur this minor variation in form of the appendages. It is not a regional variation, as all the animals examined for this purpose came from the same area. The feature of the longer third branch of the inferior appendage is not, however, as common as the shorter branch. It is not intended here to discuss the probable significance of these variations in form of the genitalia, but only to give an account of their occurence.

v. <u>General Activity.</u>

Concerning the flight of Trichoptera, there have been two important works in recent years. Corbet and Andfinn Tjønneland (1955) studied the noctural flight activity of Trichoptera and give a classification of 12 species, with emphasis on the relative development of the two activity peaks, at dusk and dawn. Collectively, Trichoptera were found to show a bimodal activity pattern, but within this, they discern 7 stages in the development of this, beginning with species which only fly at dusk. Brindle (1958) in considering the flight of the individuals taken, maintains three types of flight could be distinguished: (a) a dusk flight, (b) a night flight, (c) an intermittent flight. It is a clear cut and briefer classification than that given by the previous authors, which is given in more detail in order to give some idea of the relative development of the two activity peaks.

<u>T. obsoleta</u> adults from all observations fall into the first category given by Brindle. Flight beginning just before dusk, and continuing for one to two hours, during which time sporadic activity does occur. The main flight occurs at dusk, when the 'flies' may be picked out against the

fading sky, flying in a zig-zag fashion over vegetation beside the river, as pale moth-like forms, with their long antennae held obliquely in front of them. In the type locality of the Upper Styx where vegetation overhangs the stream, the 'flies' are active in the immediate vicinity of the water. After dusk the adults were taken with the aid of a 300 candle power tilley lamp.

During the last week in November, it was decided to check this flight activity and if possible to see whether or not a dawn flight occurred. Approximately 1 hour before the dawn broke, the Tilley lamp was lit and the net was at hand ready for the flight. Dawn broke at 4 a.m., by which time no 'flies' were seen, and by 4.30 a.m. there was too much light, so that all flight would have been inhibited (Corbet and Tjønneland 1955). Environmental conditions for flying on this particular morning were perfect, being very calm and mild, but with some slight condensation. It was therefore concluded that, because the conditions were adequate, and because no flight occurred, this was confirmation that this species may be classified as a dusk flier and therefore has a unimodal peak of activity. It should be mentioned that conditions, especially light, were so variable in the insectary, that the activity of the adults would not be normal. Because of this observations of the adults in captivity could not be depended upon for additional information. An interesting fact to note is that of the only examples used by Brindle falling into this type, all belong to the family Leptoceridae.

On warm, dry nights the adults are readily found flying in close proximity to the water. If there is too much wind the adults as a rule do not fly. On two occasions when it was wet, the activity of the adults was observed during the usual flying time. The weather conditions were adverse to flying, and the adults were found in the long grass. They were located by their movement, running through the grass with their antennae laid posteriorly along the body: 6 of these were found within a few minutes with the aid of the Tilley lamp. When disturbed they ran even further in between the grass stalks; there was no tendency to fly. Attempts have been made to find the adults in the daytime, but they have not been located. It may be that when the vegetation is swept with a net, the adults instead of flying off, run further into the vegetation. In their natural habitat the colouration of the wings is very cryptic. Even in the small insectory, it is often difficult to locate the adults when they are at rest.

Collections show that the ratio of males to females caught was 2 : 1. This may indicate that activity of the male and female differs. Females were very often found resting on vegeation beside the stream, and only rose when mechanically disturbed; they were also seen to fly very close to the water, rising and falling and then resting at the side of the stream. The males on the other hand were more commonly found in flight, several feet above the water and the surrounding area; and as these were more conspicuous, were caught in greater numbers than the females. Thus the ratio given is more than likely not a true representation of the sex ratio.

vi. Food and Longevity.

Observations made on the feeding of caddisflies are scattered throughout the literature. Crichton (1957) gives the more important of these in a survey of the published works. Dohler (1914) is reported to have performed experiments on flies in captivity, the results of which show conclusively that if caddisflies are fed on a sugar solution their length of life is considerably increased when compared with 'flies' that had been provided with water only.

Field observations have not been undertaken, but adults of this species have been kept in captivity as already mentioned, and have been seen frequently actively taking up water with haustellum protruded. The liquid must be taken up along the channels of the haustellum which converge and finally end up on the sitaphore and thence to the mouth. Two specimens were found in which the midgut was filled with a purple fluid. At the time when these particular adults were caught it was late summer and the Elderbery berries were very ripe, and it is suggested that the dark purple fluid was in fact Elderberry juice. On one other occasion a green fluid was found in the hind gut. For the remainder of the specimens examined here, however, if the gut contained anything at all it was a colourless liquid only. The nature of the gut with reference to feeding will be discussed in Chapter 3. There have been few definite records of caddis-flies feeding on nectar from flowers according to Dohler (1914) in Crichton (1957).

Once in the laboratory the water supply in the insectary dried up, and the adults were without water for a day. When water was supplied, several of the adults were observed to drink from drops of water which were placed near by.

Crichton concludes from his review that observations on the effect of sugar solution supplied to the adults in captivity and the obsorptive properties of the midgut cells indicate that some caddisflies are capable of imbibing and utilizing a nutrient solution. Observations on the species T. obsoleta definitely confirm that the adult is able to imbibe liquid, and that the availability of it has a marked effect on the longevity of the species in captivity. Whether or not it is also able to utilize a nutrient solution has yet to be examined.

Providing that the adults kept in captivity were supplied with water, they were commonly kept alive for an average of 10 days. One male was kept alive for 19 days. These observations were only incidental to the main work in hand which was concerned with the breeding of the adults. Crichton (1957) has confirmed the longevity of some Trichoptera in the field. This was done by makking individuals which were recaptured at the same site up to 35 days later.

In conclusion this species has been seen to take up water by means of a well developed haustellum; and it was found that the longevity of the species from observation in the laboratory depends on the availability of water.

vii. Copulation.

Neither copulation nor what could be definitely identified as mating flights as distinct from ordinary activity has been observed in the field. A glimpse of behaviour prior to pairing and the process of copulation has been watched in captivity. Obvious pairs in the insectory may be seen, resting on any solid substrate, frequently on stems of grass, facing each other. The female is always slightly larger than the male and the only movement seen is that of the antennae. When at rest the antennae always lie along the substrate in front of the animal; when in pairs, however, the antennae are held slightly erect and are used as feelers. It would seem from the brief examinations made of mating behaviour, that the antennae play an important functional role as sensory structures in pairing.

In copulation the male and female come to lie so that their heads face in opposite directions and the genital segments are apposed. The female's wings lie roofed over those of the male. One pair was seen 'in copula' for 1 hour, no other times have been taken. The inferior appendages during copulation act as efficient claspers. This was observed when the pair were resting on the inside of one of the collecting jars and could be readily seen by the use of a binocular micrscope fitted on to an arm which was moveable on an upright stand. The lateral sclerotised lamellae of the female were enclosed in the genital pouch of the male, while the superior appendages were held outside, and appeared to act as subsiduary claspers.

It has been noted by Despax (1951) that in general it is in flight that the male searches for the female. The sex ratio which was given in section v. may indicate that this is in fact the situation we have here, the females on the whole being less active in mating activities than the males.

viii. Oviposition and the Egg Mass.

Oviposition has unfortunately not been observed. Frequently females were seen flying close to the banks of the stream and it is thought that they were probably in search of suitable laying sites. The habitat of the egg mass is not known; some information has, however, been obtained from adults kept in the insectary. On the floor of the insectary was placed a flat dish containing water about $\frac{1}{2}$ inch deep. The dish was supported at one end so that the water only covered a small portion of the bottom surface. Some vegetation was arranged so that some of it was submerged. Thus the eggs could be laid at the edge of the water, on the submerged vegetation, or deposited freely on the water as is known for some Leptoceridae, (Despax 1951).

The dish was frequently investigated, and finally it was found that one of the females had affixed two egg masses to the bottom of the dish at the edge of the water. As in all the Leptoceridae the egg mass was of the gelatinous type, as opposed to the cement type of egg mass. The egg mass was irregularly convex and each measured 5 x 4mm and 4 x 3mm respectively.

The egg measured 0.71mm x 0.560mm and the yolk 0.310mm in diamter. The expansion of the egg membrane had presumably come about by the absorption of water. The number of eggs in each egg mass was seven and ten. This is only a very small percentage of the total number of eggs found in the ovary of one female, so it may be that one female in fact lays several egg masses. Sand grains and other forms of detritus soon become adhered to the gelatinous mass.

# Chapter 2 : SOME ASPECTS OF LARVAL LIFE.

## 2/1 The Case and Casebuilding

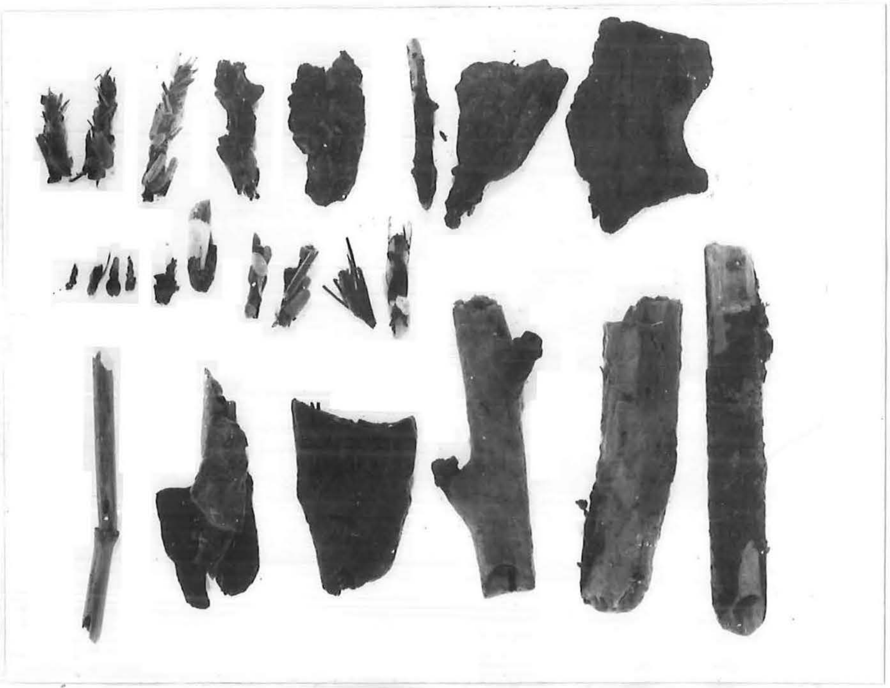
### i. Introduction.

After some preliminary investigation it was found that the form of the larval case was extremely diverse: it is, however, nearly always constructed from plant material. The variation in material used was especially noticeable between the larvae of the type locality and from the Lower Styx area. Also it was noted from observations made that the material used in case construction varied with age of the larva. In order to further substantiate these observations, collections of 100 larvae were made from each locality, for the months of May, June, July and August. Immediately the larvae were brought into the laboratory they were placed in 70% alcohol. Length measurements and head width measurements were made of the larvae and correlated with each of these the case composition was recorded.

Samples of larvae were kept in the laboratory in small aquaria and given only certain types of material for case construction. This was in order to determine whether or not the larvae were at all selective in the material used for case building.

Many observations on the different aspects of case building by the caddisfly larvae have been made. Hanna (1960) describes eight main methods of starting a case, as seen in a number of species, and he goes on to describe special adaptations of the legs so that material is easily manipulated in case building. Hanna (1961) says that little work has been done on the selection of materials for case building by caddis larvae. The majority of published observations are brief and unsupported by detailed experimental work. His work on the selection of materials for case building is both qualitative and quantitative, and his results show that the quality, size, and shape of the material are the three main factors that play an important part in its selection for building cases. Tindall (1960) gives an interesting description of the case of Triaenodes bicolor Curtis. and some special adaptations of the limbs for case building, together with the likely reason for the asymmetry of the mandibles which overlap when closed, thus providing an efficient locking device well suited for tearing away tough plant fibres. Dodds and Hisaw (1925) in their ecological studies on aquatic insects give a section on the adaptations of caddis-fly larvae to swift streams. In this they suggest it is the case rather than the body of the larva which exhibits the peculiarities of form which fit each species for its type of environment. The light, bulky and more cumbersome cases being built by larvae inhabiting slow running streams and lakes, while in swifter waters the case is more advantegeous if built of heavier materials, at least heavy enough to sink if the insect becomes dislodged.

The form of the cas e built by larval Trichoptera is extremely diverse and the material used in their construction vaires considerably depending largely upon the type of habitat in which the larva lives. Sand grains, sedges, plant foliage, pondweed and other types of plant debris are very commonly used in case construction. Also some larvae secrete a horny substance which is fashioned into the very smooth, cylindrical case characteristic of Olinga feredayi. Often the pieces of plant material to



1cm.

Fig.36a Larval cases from the Upper Styx River

1 cm.

1 cm

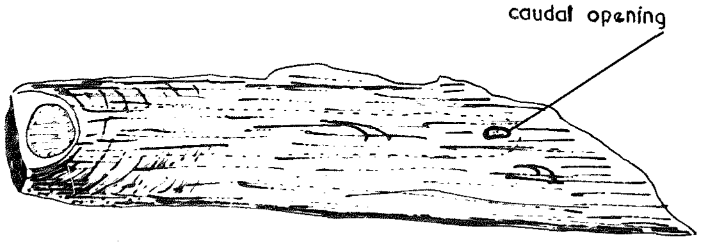
Fig.36a(above)&b(below) Larval cases
from the Lower Styx River.

be used in case construction are cut by the mandibles to a definite size and shape before being attached to the case by the silk thread; the resulting case is always of a characteristic shape. In other instances the material is not cut to a definite shape and may overlap, to a greater or lesser extent, the remainder of the case, so that a more bulky form of case results. The interesting feature about the Triplectides obsoleta larva is that besides employing the second method of case building stated in the previous sentence, the larva frequently fashions its case by boring into solid twigs and using them for cases. This feature has been recorded to my knowledge only once before in the literature, by Lloyd (1915). These two main types of cases and the factors which govern their distribution will be discussed in the succeeding subsections.

ii. Description of Case Structure.

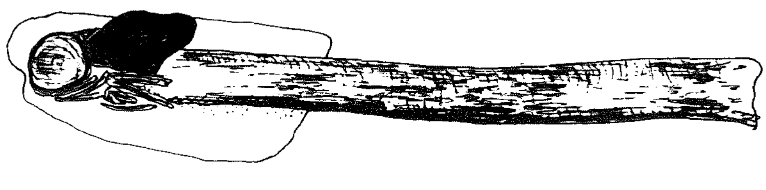
A great variety of plant material is used by the larva of T. obsoleta for case making. The two main types of case, a) that composed of an assortment of material, chiefly of plant origin, only occasionally of sand grains; and b) a case made by tunnelling into solid twigs and pieces of bark of suitable size and texture.

When the case is built of assorted material it is usually wider anteriorly and tapers slightly towards the caudal opening (Fig.36 a ). Small pieces of wood, willow leaves, seeds, Elodea leaves and fine twigs about the size of root fibres are all incorporated into the case, in varying proportions. It is normal for the case to be composed of a mixture of these types of plant material (Fig.36 a,b,c). When the case length exceeds about 15mm it is very often found to have one or two main supports




a. hollowed wood type of case.

b. case made from hollowed wood with material added at the caudal and cephalic end.



c. hollowed type of case extended at the cephalic end.

Fig. 37

(Fig.36 b and c, top rows), the support being a single piece of wood attached along the longitudinal axis of the case. In some instances the support may be as much as three times the length of the case itself. The pieces of plant material are not arranged in any particular fashion when being incorporated into the case, as can be seen from Figure 36 a,b, and c. The young larvae in most areas build cases of assorted material. Sand grains are seen frequently in the tiny cases of 2nd instar larvae.

During the Autumn when seeds from the Elderberry in particular were present, many of the cases of assorted material were either partly or sometimes completely made out of seeds. These were common in the Upper Styx samples, but were occasionally seen in the Lower Styx material (Fig.36 c bottom row).

The case made from a solid piece of wood, either a twig or a flat piece of bark (Fig.36 a) is not always occupied entirely by the larva, which frequently lives only in a third or even less of the total length of the wood. When the larva does not occupy the whole length of the wood it makes a contact with the exterior by cutting a small opening on one side of the twig (Fig.37 a) so that a through current of water is still maintained. Sometime later after the larva has increased in length the solid portion of the stick may be hollowed out and the caudal opening is then shifted to the posterior end of the twig. This is more commonly the situation. Around the cephalic and caudal openings of these cases the larva often adds small pieces of wood and other material (Fig.37 b). The length of the case is sometimes extended by the addition of such material to the anterior end (Fig.37 c).

Cases made from assorted material and also the ones made from hollowed woody material, are lined with a very closely woven silk cocoon, secreted from the aperture at the tip of the labium. The thread is of a double nature, and the lining forms a complete cocoon which may be dissected out whole from the case material. Bits of organic and inorganic material readily adhere to any exposed parts of the silk cocoon, so that there are seldom any gaps between the main pieces used in the case construction.

iii. Observations made on the construction of the two types of larval case.

When larvae were taken from their cases and placed in a petri dish with a supply of small fragments of wood, etc. they began, after a brief time, to sort out the material and to construct a new case. This process was watched several times and the larvae seemed to employ the first of the eight alternate methods that Hanna (1960) states as being the main methods used by larvae starting new cases. This is a simple method whereby four sides are constructed around the fore part of the larva and material is then added in front, so that gradually the case extends around the larva from before backwards. The first pieces of the case are strung together by the silk thread emitted by the silk duct opening on the tip of the ligula. The maxillo labium is seen to flicker over the surface of the material to be used, and as it does so the thread adheres to the surface. Then by passing back and forth from the initial piece of material to the piece being incorporated, the two are joined by the criss-crossing of the silk thread.

The pieces are held mainly by the angle between the femur and tibia of the prothoracic legs. The metathoracic legs wave around and when they

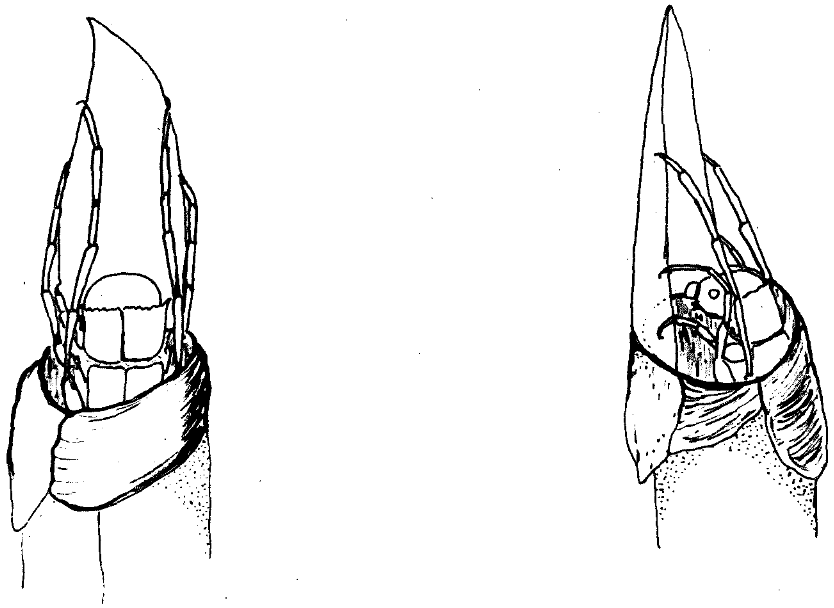


Fig.38.a. To show role of legs in the addition of material in case construction.

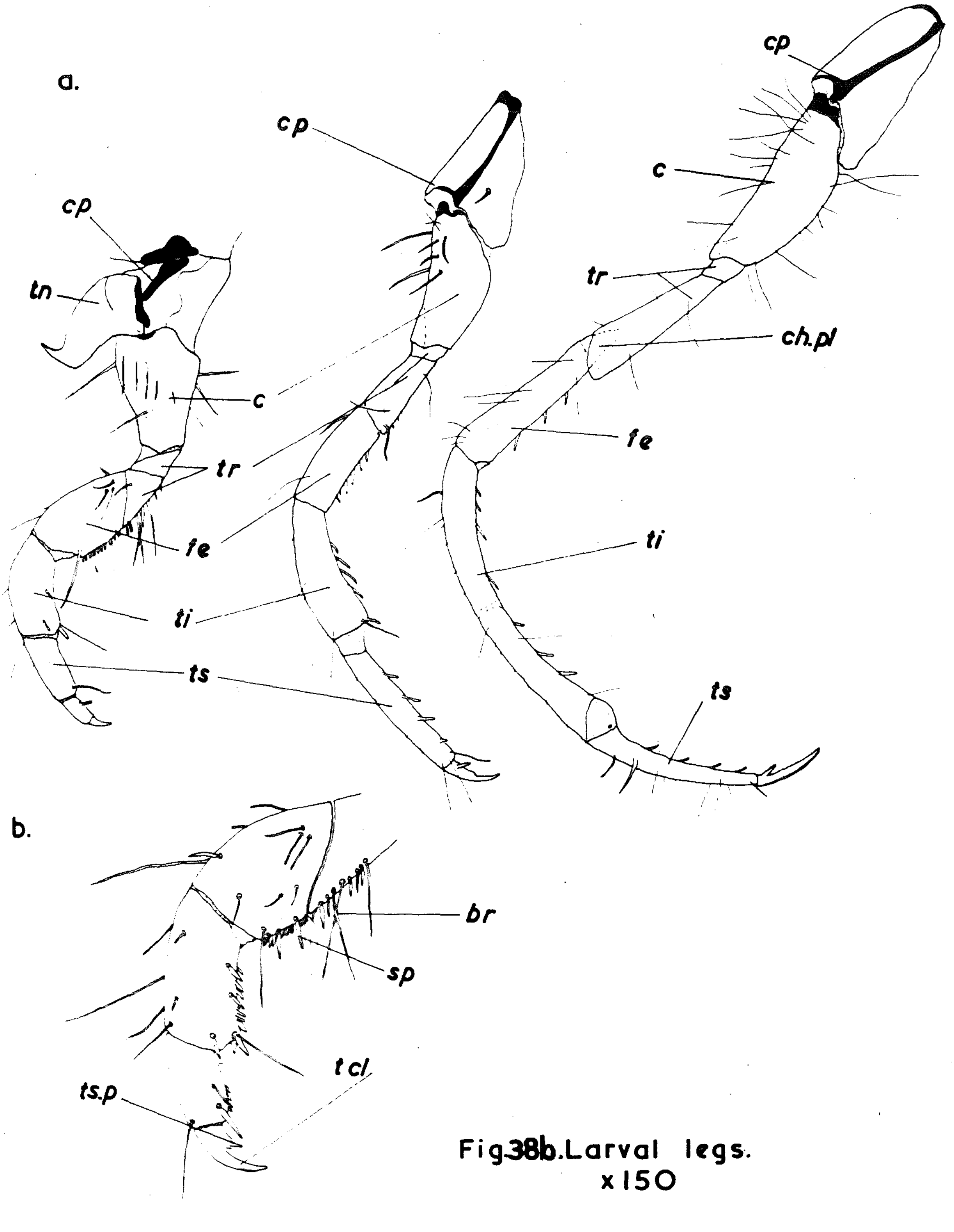



Fig38b. Larval legs.
x150

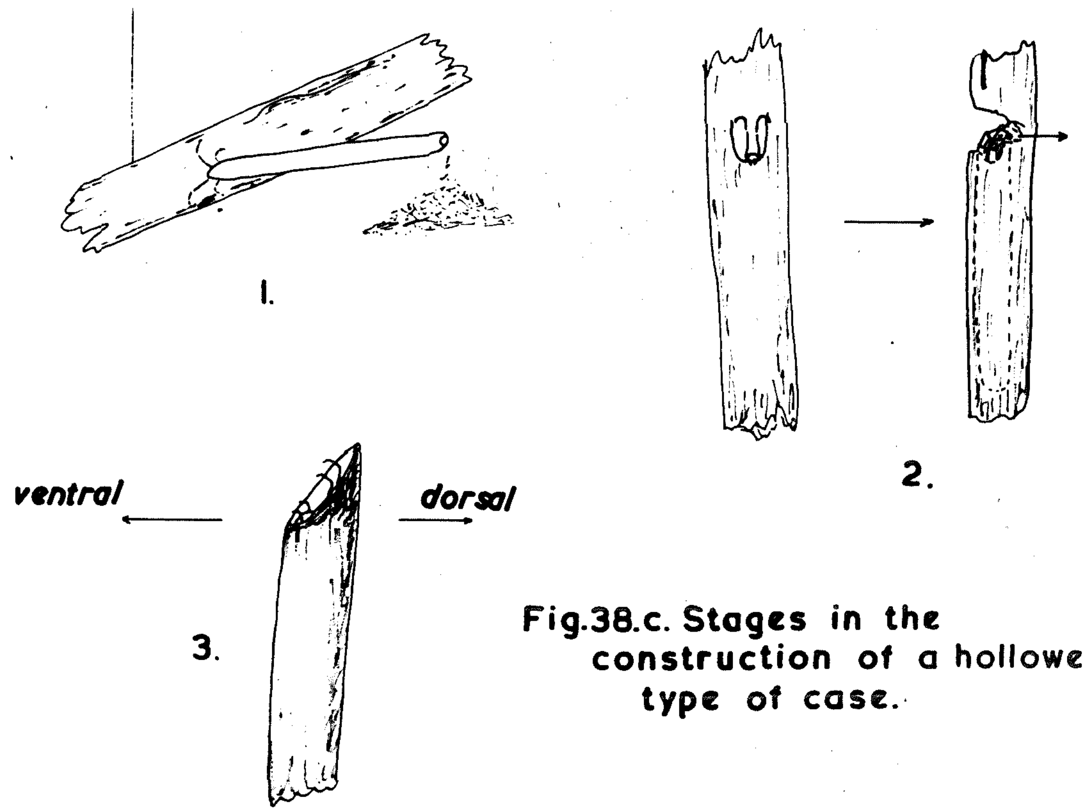



Fig.38.c. Stages in the construction of a hollowed type of case.

contact a likely piece of material it is pulled into the pro and meso-thoracic legs by the tarsal claws. Succeeding pieces are added to the case, each individually being held in place chiefly by the pro and meso-thoracic legs, the latter holding onto the edges of the object with their tarsal claws. If the object is long then the tarsal claws of the meta-thoracic legs are also used (Fig.38 a), to hold it in place. The head is moved back and forth from one piece to the next until each succeeding piece is firmly fixed to the remainder of the case. Then finally, when the case is long enough, the larva adds more silk to the inside of the case so as to form a more compact lining.

Hanna (1961) says that since "the material used in the construction of the case is gathered and manipulated by the legs it would be expected that these should show adaptations for this activity". He goes on to cite some of the different ways of holding the material while incorporating it into the case, and some of the corresponding modifications seen. In the T. obsoleta larva it was found, as already indicated, that material is held between the angle of the femora and tibiae of the prothoracic legs and also by the claws of the mesothoracic and sometimes metathoracic legs. Fig.38 b shows that there is an indentation on the inner surface of the angle between the femur and tibia of the prothoracic leg. Associated with this are well designed spines; the one on the distal end of the femur especially, would aid in the grasping of material to be incorporated into the case. The outer edge of the fragments of wood and other materials are made more easily held by the above indentation. The spines are present also on the inner margin of some of the segments of the meso- and meta-legs

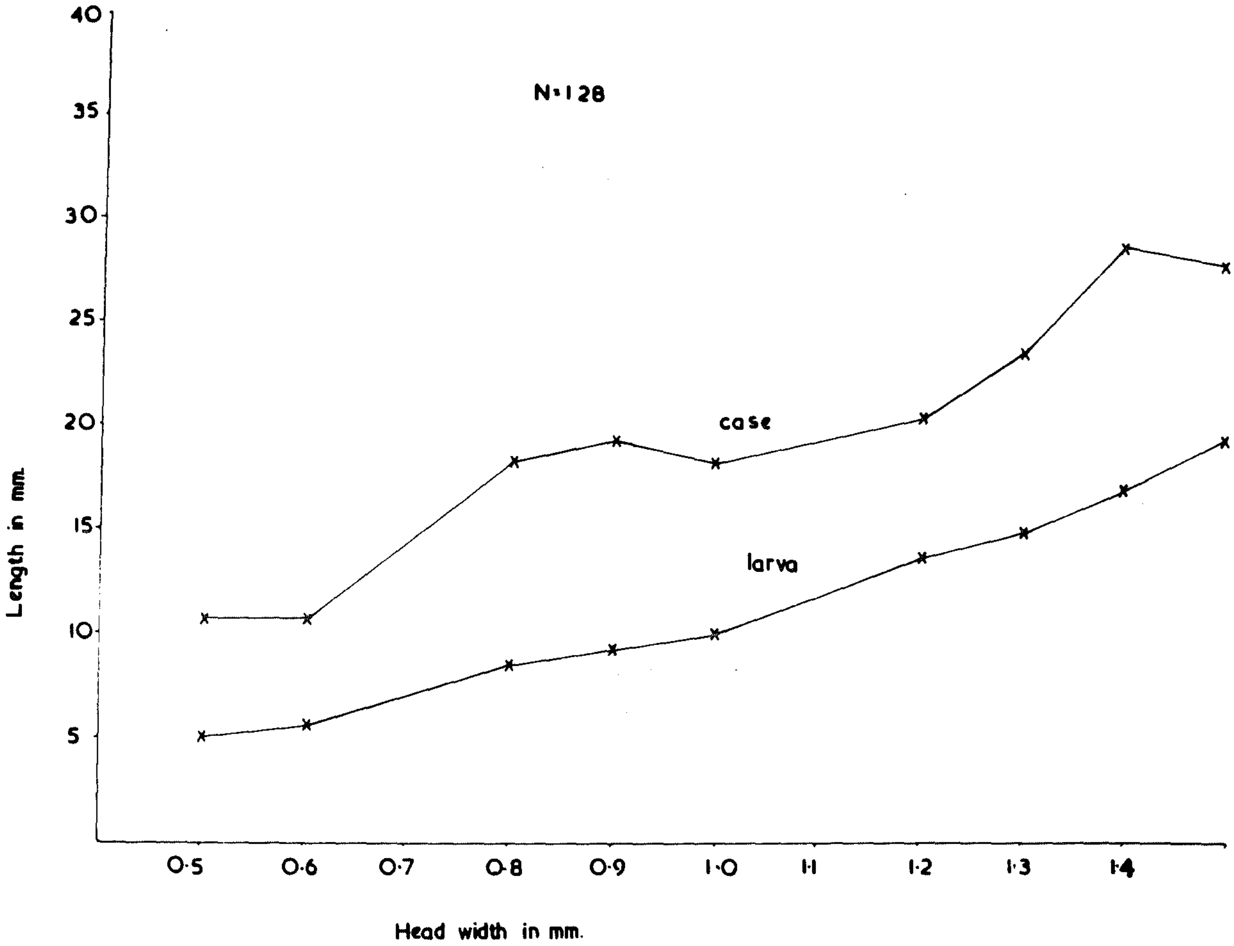



Fig.39. Correlation of growth of larva with growth of case.

although they are not so well developed, together with the tarsal claws (Fig.38 b t.cl.) these spines probably aid the efficiency with which the meso and metathoracic legs pull in the material for case building. The spine (Fig.38 b t.sp.) present at the base of the claw of all three legs probably also aids in grasping and holding objects. Hickin (1946) says of the eruciform and suberuciform larvae "that there are often a series of spines on the ventral edge of the femur which are often strong and of two distinct sizes, so that when the tibia and tarsus are pulled against the femur by the strong muscles which are present, the prey is very efficiently trapped". This would seem to be the situation in this species with regard to objects used for case building.

The diameter of the case is increased as the larva grows, and that part of the case which is no longer required posteriorly, is cut off by the mandibles. The larva reverses its position in the case to perform this function. In this way the case is kept from becoming too long. As Hanna (1957) found, so it was seen here, that the growth of the case takes place parallel with the growth of the larva (Fig.39).

When the late instar larvae find a suitable solid object to make a case, some of which are seen in Fig.36 a, a slight hollow is formed on the surface by the action of the mandibles described later, while the claws of the legs hold the larva in position. Then the old case is very firmly attached by silk threads (Fig.38 c,l.) and the tunnelling proceeds. Fine wood shavings are wafted out the caudal opening of the case and as the larva bores deeper so the pile of shavings grows. In one particular instance it took $1\frac{1}{2}$ days for the larva to hollow out a cavity long enough to accomodate itself. Once this was done, the connecting silk threads between the old and new case were severed by the mandibles, so freeing the old case.

The new case was then lined with silk and contact made with the exterior at the posterior or caudal end of the case; either at the side or through the end, depending on the length of the stick. Frequently the larva begins to fashion itself a new case by going in from one side of the object and not from one end, consequently there may be a varying length of stick anterior to the cephalic opening (Fig.38 c,2.). This is then removed by the larva, which cuts a transverse incision across the long axis of the wood. Finally the larva gives a dorsal and ventral side to the case by tearing away at one side so that this will become ventral (Fig.38 c,3.).

The mandibles which have been described in Section 1/1 are so articulated that when closed the tips overlap with one another, thus providing an excellent interlocking device for tearing away tough woody fibres. This mechanism has been also described by Tindall (1961) for Triaenodes bicolor, which also belongs to the Family Leptoceridae. While in the process of burrowing into wood, the movement of the labrum and the maxillolabium with their respective brushes of bristles, probably play an active part in clearing the shavings, away from the vicinity of the mandibles. Thus enabling the through current of water to carry the shavings past the body and out the caudal opening.

iv. Selection of material used for case building according to habitat and age of the larva.

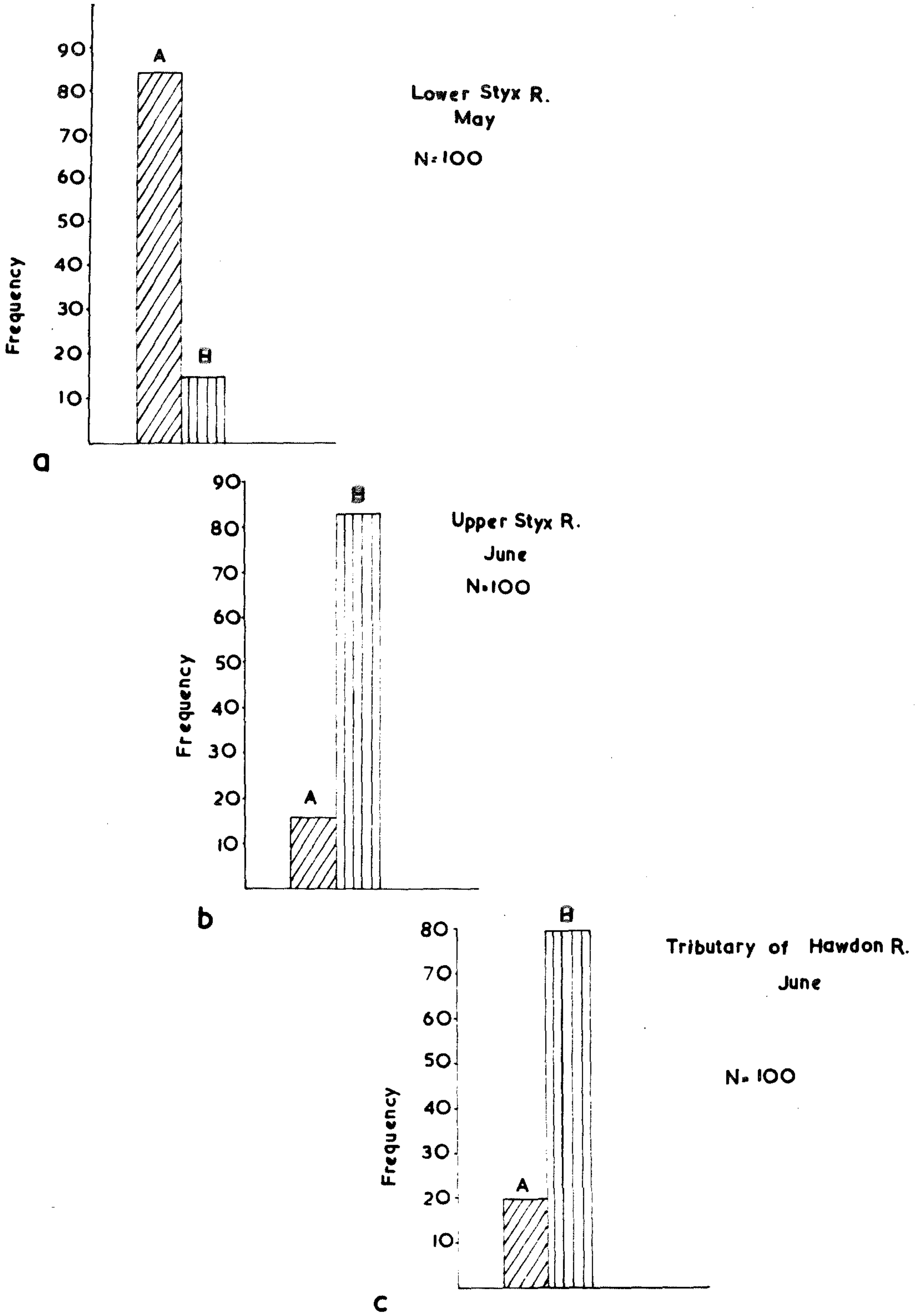



Fig.40. Material used in case construction in three different localities.

A. assorted material.

B hollowed woody material.

Results obtained from collections made in the Upper Styx and Lower Styx areas (Fig.14) show a definite difference in the quality of the materials used in case building. Larval cases from the Lower Styx are mainly composed of assorted material, only a few cases are made from hollowed out woody material (Fig.40 a). Cases taken from the Upper Styx area show the opposite trend, there being a predominance of hollowed sticks used for cases (Fig.40 b). A third area, a tributary of the Hawdon River near Cass, had two main samples taken during the winter months and the results of these show that here also the hollowed stick is the commonest type of larval case (Fig.40 c).

To find a reason for these obvious differences in case types we must look at the habitat of the larvae in these different areas. In both the tributary of the Hawdon and in the Upper branch of the Styx River the streams are supplied with a great amount of plant debris which is in very close proximity to the water. The type of debris here consists of fallen logs, an abundance of broken sticks and slender twigs, which are the appropriate materials for the hollowed cases of some of these larvae, together with a very wide assortment of other plant debris.

In the Lower Styx, on the other hand, the river is not overgrown in the same way with vegetation. The river is deep and very much wider, the willows line the river, but do not overhang the water to the same extent, and consequently there is less in the way of litter falling directly into the water. Also larvae taken from this area were swept from the ~~sides~~ Elodea along the sides of the river where there is little accumulation of plant debris. The cases of these larvae usually consist of bits of willow

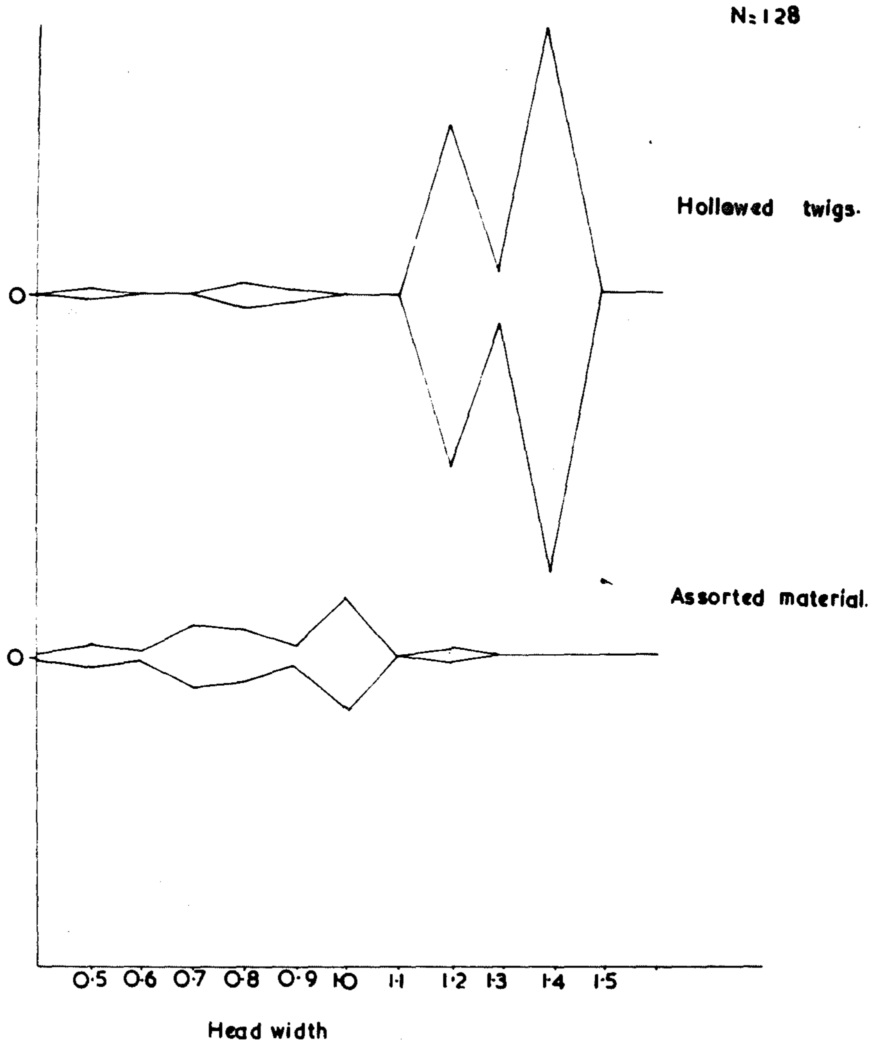



Fig.41. Correlation of the two main types of case construction and selection of these with age.

leaf, Elodea stems and other light vegetable material, all readily available at the side of the river. It would seem that here the available material was not particularly suited for making cases out of solid pieces of wood and so an alternate method was found. In the Upper Styx and in the tributary of the Hawdon, the material was of a different nature and accumulated where the larvae could reaily utilize it. The remarks made earlier in the introduction from Dodds and Hisaw (1926) may possibly have some effect here. The Upper Styx, being a faster flowing stream than the more slowly flowing Lower Styx River, so that the majority of the larvae build cases from solid objects, these being less likely to float and readily sink when dislodged.

Before however any conclusion can be reached concerning this preference for using twigs etc if available for case making, the question of preference according to age of the larva must be considered.

In samples taken from the Upper Styx where all types of debris are available, it was found that there was a marked tendency for most of the early instars, from the 2nd to about the 4th, to build cases from assorted material; while from approximately the 4th to the 6th instars had cases mainly made from hollowed out pieces of wood. This is shown very markedly in one sample taken in August (Fig.41). It is important to remember that the 5th and 6th instar larvae were more abundant during this month than preceding months, which accentuates the tendency to a marked degree, by the greater frequency of these instars present in the sample. However, earlier on in the year the case structure was correlated with larval length and although the results differ in that the predominance of numbers with

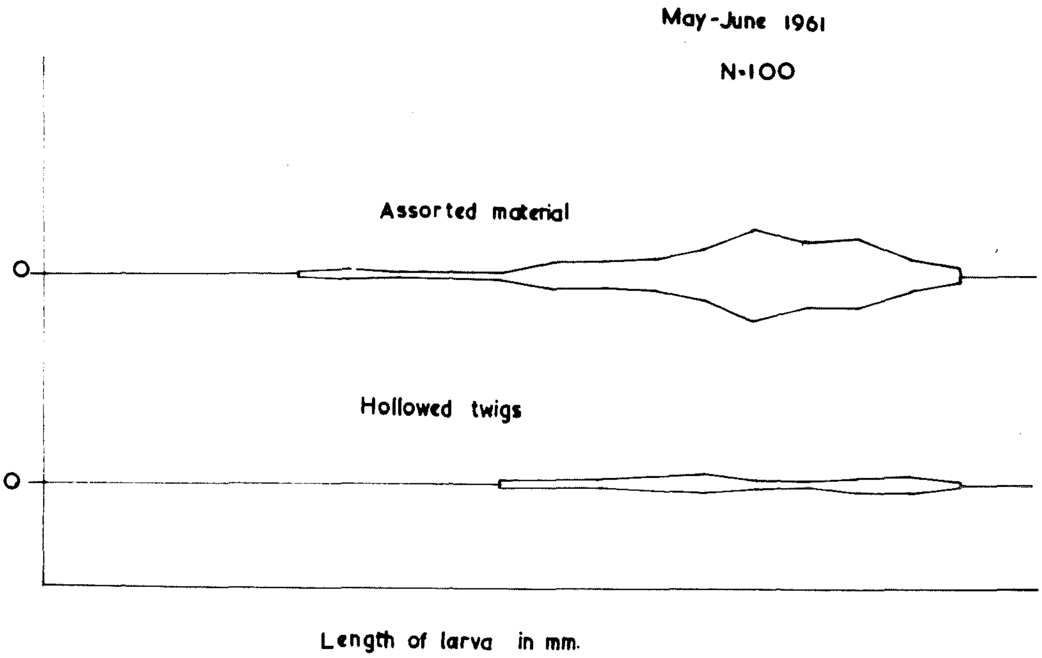



Fig.43. Correlation of larval length and case composition. Lower Styx area.

hollowed cases is not the same, yet it is obvious from Fig.42 a and b that although the younger larvae may show a slight tendency to hollow out solid pieces of wood, the majority use assorted material in case making. The older and longer larvae, although not in such great numbers during April and May, can be seen to make cases by their boring ability. At the same time there will always be a very few of these last two instars building cases of assorted material.

In the Lower Styx samples, where the assorted material predominates in case structure right throughout the larval life, the hollowed woody cases if represented are normally seen only in the older larvae. Unfortunately head width measurements were not related to case structure in the samples taken from the Lower Styx but the general concept can be readily see in Fig.43 where only the length of the larva was related to case structure.

From these results it is evident that age of the larva has an effect on the selection of material for case building. That there is a preference for boring into woody material by older larvae for case construction, is seen clearly from results of the Upper Styx samples. In the absence of this material, however, the larvae of these last instars can build cases of assorted material. These cases are correspondingly more substantial compared with those of the early instars (Fig.36 a, b, c.). It is thought that there is an obvious advantage for the last instars in particular, to construct their cases from solid woody material. This type of case appears to have more lasting qualities than that made out of assorted material. In the latter case the larva frequently has to repair parts of the case and also add pieces to accomodate for increase in growth. Also cases built from live material such as Elodea leaves have to be added to very soon, as the leaves die and give no support to the case. Therefore from the point of view that the last two instars are normally at these respective stages for a longer space of time than the early instars, the hollowed out piece of wood seems a more suitable type of case compared with the assorted type of case structure which is probably less durable.

v. Selection of materials for case building, as seen from observations made in the laboratory.

A brief study on the selection of materials by the larvae for case building was made. Four different samples of larvae were placed in small aquaria, measuring $5\frac{1}{2}$" long by 4" wide and $2\frac{1}{2}$" high. These were half filled with water which was maintained at the same level throughout the time of observation. Each aquarium was kept well supplied with the pond weed, Elodea sp., a fresh water snail, Potamopyrgus sp., and the water was not aerated by any mechanical device.

In the first aquarium there were four larvae with cases built of assorted dead plant material, the lengths of which were characteristic for 3rd and 4th stage larvae. They were provided with Elodea sp. and small pieces of wood. After one month a single solid twig was placed in the aquarium, and upon examination two weeks later the 4th instar larva had hollowed this out for a case. The remaining larvae, as before the addition of solid wood, only added small bits of bark to their cases. Further solid material was added, this was ultimately used by the larvae for case material. A week later a second larva had hollowed out a length of wood for a case, and by the end of a further week a third larva had done the same thing. Only one larva retained its case of assorted material by the end of three months.

In the second aquarium there were three 2nd instar larvae, whose cases averaged 5mm in length and were composed of fine root fibres and other dead plant material. The only other plant material in the container was some Elodea. By the end of one month each case was composed almost entirely of Elodea leaves, at this juncture some solid twigs and pieces of bark were added. By the end of a further two months the cases were composed chiefly of an assortment of dead plant material. There was no indication that the solid material was hollowed for case building, or that live material was used in preference to dead plant material, when the latter was present, rather the dead plant material seemed strongly prefered to alive plant material.

The third aquarium contained larvae of 3rd and 4th instars and had cases composed of assorted material both living and dead. The case structure did not change very much throughout the three months observation. Twigs and pieces of bark were present, but the larvae used only small bits of bark to extend their cases.

The fourth aquarium contained four larvae of the 4th instar stage, all with cases fashioned out of hollow twigs. There was no dead plant material in the aquarium. One of the larvae as a result of this fact could only lengthen its case by incorporating Elodea leaves on to the cephalic end of the case. After some sticks and bark were added the larvae accomodated their increase in length by hollowing out further cases from this woody material. Length of the case was not seen to be increased by the addition of fragments of material etc., providing that solid material was available for tunnelling out a new case.

Unfortunately these larvae did not survive for any longer than from 3 to $3\frac{1}{2}$ months, after which time all the larvae had died, probably owing to the conditions in these small containers. However the observations that were made together with results from field samples are adequate for some conclusions to be made concerning selection of material for case building.

iv. Conclusion.

The use of sand grains in case building was not closely investigated, but these were found to be used only in the early 2nd instar larvae. Occasionally the whole case was composed of sand grains but more often the anterior half was made of plant material while only the posterior half was composed of sand grains. The late 2nd instar larvae has a case composed almost entirely of plant fragments. It is concluded from these observations that the case of the 1st instar larvae will have cases constructed out of sand grains. Finally it may be noted that the later instar larvae, especially the 5th and 6th instars, show a preference for building cases from solid pieces of wood which include twigs or small sections of bark. In the absence of this material however, the larvae continue to use assorted pieces of usually dead plant material, which is the normal material used by the early instars. These latter instars show only a very slight tendency to fashion cases from solid woody material, their cases being more of a temporary nature than those constructed by the older larvae.

## 2/2 Food and Feeding Habits of the Larva.

### i. Introduction.

A preliminary investigation of the feeding habits and the nature of the food organisms taken up by the larvae has been made. These studies however have not been undertaken throughout the whole year nor were they investigated for each instar. Considering the seasonal variation in the abundance of food items and also the probability of differential selection of food material by the larva; a study of this aspect of larval life would not be complete unless investigations were made for one year, together with a consideration of the variation which might occur in the diet of each instar. Finally it is difficult to determine food items which are actually digested and used to nourish the larva.

Hanna (1957) reviews the literature concerning the subject of the food of Trichopterous larvae, and concludes that most of the works were done over a short period of larval life and that none of them considered the possibility of seasonal variations in the diet of the larva. In order to obtain a more accurate account of the true situation Hanna gives a quantitative study of the feeding habits of four species of caddisflies throughout their larval life. Also he found evidence that there was a substantial increase in the abundance of food items in the midgut which could be associated with an increase in rate of growth.

In the larva of Triplectides obsoleta the observations made on the feeding habits led to an investigation of the exact nature of its diet, and ultimately connected with this an attempt was made to determine the food items actually digested by the larva. Finally a brief mention is made concerning the correlation of feeding habits with morphological characters.

ii. Observations on the feeding habits of the larva.

The larvae are normally found crawling over woody substrates, in amongst the pond weed Elodea sp., along the sides of the stream, among the roots of grasses and other material, sometimes over stones on the stream bed, when they occur, and very commonly they are found in the surface debris of fallen leaves and other plant debris. A closer observation in order to determine the purpose of this activity was carried out. The larvae were placed in glass dishes, covered with water and provided with a variety of material, including Elodea and dead plant material. The larvae were then observed through a binocular microscope fitted on a moveable arm.

The larvae were seen to browse over the surface of the wood probably covered with a film of microflora and fauna. The mouthparts appeared actively to scrape the surface and frequently the mandibles would tear off portions of the woody substrate. When the larvae were not feeding the maxillo labium was often seen protruding and seemed to be feeling the surface for further food. Pieces of wood which have been in aquaria with larvae for any length of time, are seen to have numerous small concavities over the surface, indicating where the larvae have been feeding. Some of these bits of wood after a number of months were found completely stripped of the outer layer of bark, due to the activity of the larvae. The larvae were also observed browsing on the surfaces of dead leaves in the same manner; more especially the early instars were characteristically found on these leaves.

A second method of feeding besides this one was observed. Larvae were seen actually tearing small pieces of tissue away from the leaves of

Elodea with their mandibles. Evidence of this was also noted when fresh Elodea was placed in with the larvae. This, after varying lengths of time depending upon the feeding habits of the larvae present, was observed to have small notches cut out of the margins of the Elodea leaves. The behaviour of the larvae when on these leaves suggests that here also they scrape the surface film from the leaves.

iii. Examination of the gut content.

The alimentary canal was dissected from the larvae to be examined. It was cut into three parts, the fore, mid and hind gut, with a sharp razor blade; each portion was placed in a covered solid watch glass with a little distilled water. When required for examination under the microscope the gut content was pressed out, and a drop of this was then placed on a clean dry slide. All the instruments and glass material used were boiled and kept as free from contamination as possible.

The results obtained were from larvae freshly collected in June and July. The abundance of food organisms and also the occurrence of these, was noted to vary in the different individuals, although no quantitative evidence is available. It is difficult to compare the relative abundance of each food item in a single gut content sample, since there is a marked difference in the size of the different components. However, it can safely be said that the bulk of the food present in the alimentary canal is dead plant tissue chiefly woody fibres. Normally the midgut was found to be packed from end to end with these woody shavings of fibres and cells. Only seldom were these seen in the foregut, indicating that they were passed through fairly quickly to the midgut. The hind gut also contained these woody plant cells, but they were by this time of a slightly finer texture, probably due to mechanical breaking up of the cells. Finally in the rectum the cells were seen to be the main components of the faecal pellets. These faecal pellets were not formed into a characteristic shape but were merely an irregular clump of empty plant cells which breaks up soon after they leave the larva.

Of the remaining contents of the alimentary canal the next most commonly occurring organisms were diatoms mostly belonging to the order Centrales. These were recorded from the foregut and the midgut; in the latter and in the hind gut and also in the faecal pellets it is only the case of the diatom that is found. Green cells of the pond weed Elodea were seen in the fore and midgut, and to a minor extent some empty cells of filamentous algae were noted.

Using oil emersion and with a magnification of x 1,500, numerous bacteria and unicellular green algae were seen in the fore, mid and hind gut. Finally a close examination was made of the woody plant cells of the midgut, and penetrating between the cells was commonly found fine fungal hyphae.

Without undergoing a physiological investigation it is impossible to finalise which of these food items is digested. On one occasion live cells filled with green chloroplasts were found to have bacteria which had entered the cell. The role of these bacteria in digestion and nutrition however must remain uncertain in the present work. They may of course be purely incidental in that their presence is due to being taken up with the food of the larva.

It was hoped that by the investigation of the contents of the fore, mid and hind gut, and by comparison of the state of the contents in each part an idea of the contents actually digested might be gained. From the fact that no empty cases of diatoms were found in the foregut, although these were common in the midgut, it seems likely that these were digested. Empty Elodea cells, plus various empty cells of filamentous algae had probably also been previously digested.

It is difficult to ascertain the significance of the large quantity of dead plant tissue which is eaten by the larva. It would seem very likely that it is not these dead cells themselves but rather the microflora that is found on and within the cells that is of importance to the digestion and nutrition of the larval stages of this species, which may be classified as herbiverous forms feeding mainly on detritus.

iv. Correlation of feeding habits with morphological characters.

Several authors have noted that there is some correlation between the type of mouthparts of the larvae and the food normally taken. Ulmer (1902 b ) as quoted in Davis (1934) suggested that in general larvae with blunt toothed mandibles and those with sharp teeth might be regarded as phytophagus and carnivorous respectively. Siltala (1907) as recorded in Davis (1934) regarded the presence or absence of median bristles of the mandibles as indicative of feeding habits. "He shows that all forms that have the median bristles on both mandibles are, so far as known, phytophagus, that those which lack these bristles are carnivorous, either exclusively or as much so as herbivorous. Those which have bristles on the left and not on the right mandible vary, some being carnivorous, some phytophagus and some omnivorous."

Slack (1936) applies these groupings to larvae he examined and comes to the conclusion "that the occurrence of mandibular brushes would seem to denote the quality of the food material rather than its identity"; the probable function of the mouth brushes being to carry all types of small organisms into the mouth. In T. obsoleta as seen in the previous chapter there is a brush of bristles on the left mandible and the labrum and the base of the inner lobe of the maxilla have each a well developed cushion of bristles. The mandibular brush as Slack suggests probably carries fragments of food to the mouth; as the mandibles close the brush would scrape the inner surface of the right mandible, which in the closed position (Fig.4 d) lies beneath the left mandible. The remaining bristles of the labrum and maxillo labium have been mentioned previously to act as a cleansing device, when the larva is tunnelling into wood. These could, when the larva is feeding, act equally well in the opposite direction, by the action of the muscles operating the mouthparts, and thus guide the detritus into the mouth.

By some, it is said also that the form of the mandible gives an approximate indication of the feeding habit. A large molar surface being indicative of a diatom feeder, carnivorous forms have sharply pointed faces, while the omnivorous forms and those whose food requires tearing or cutting show a development of three to five denticles. So it is with this species that the tearing action required for tunnelling and also for feeding on dead plant tissues is adequately performed by the overlapping denticulate mandibles.

The position of the eyes in this species is slightly more anterior than posterior on the head, about midway in the series figured by Wesenberg-Lund (1911 c) recorded in Davis (1934), where he is reported to have found that the more completely the animals are carnivorous the farther forward the eyes are placed. Presumably in a carnivorous feeder the eyes are important sense receptors of movement and are more efficient, if placed in an anterior position. It is not considered that the position of the eyes in this species has any definite correlation with feeding habits. It may be said however, that if the Trichopterous larvae are considered as a whole then the position of the eyes are indicative of extreme cases of a carnivorous or a phytophagus habit.

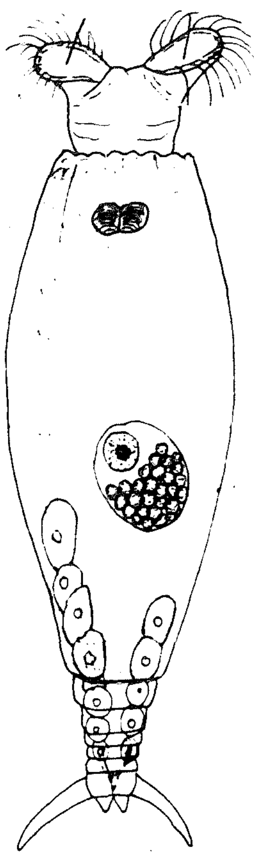
v. Conclusion.

In conclusion it may be said that statements made concerning the relation of morphological features to feeding habits as written above are by most workers applied to the group as a whole and can not with any assurity be shown to fit any single species. With T. obsoleta it has been noted that the correlation of morphological features with feeding habits lies in the form of the mandibles and the development of bristles on the mouth parts.

2/3 Symbionts.

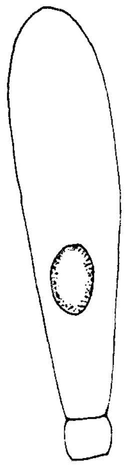
Finally in this section some mention should be made of the Rotifera and Protozoon fauna which has been fairly constantly found, occurring in and on the larval stages of this species. The term symbiont has been used in the sense defined by Allee et al. (1951), according to whom there are three main types of symbiosis; parasitism, commensalism, and mutuatism. If neither associate is harmed and at best only one benefits, we are dealing with commensalism; if both associates are benefited, the relation is mutualism; if one is harmed it is parasitism. The protozoa and rotifer found associated with Triplectides obsoleta larvae may be termed commensals. To quote Allee et al., a commensal "may be quite unattached to its host, living in close and direct association with it; it may live upon it; or it may live actually within the body of the host, in respiratory or alimentary tract or in any other cavity of the body open to the exterior". The commensals in this species are, a rotifer, a gregarine and sessile ciliates, chiefly vorticellids.

The only record found of rotifers commensal with Trichopterous larvae was that made by Scherren (1897) who notes that besides living as a commensal on Gammarus pulex and Asellus grandis, Callidina parasitica is also commensal on Phryganea grandis. Another rotifer Rotifer tardus was also found among the materials of the case. The rotifer found as commensal on this species is of the bdelloid type (Fig.44 a), moving along the surface by means of a looping method; the anterior end of the body stretches forward, and holds on to the substrate while the forked foot loops to a position just behind the anterior end, which either moves about

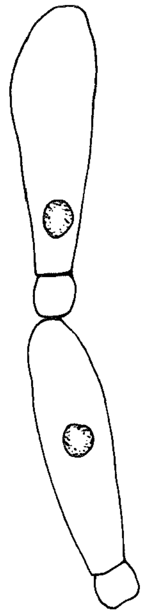


a. Rotifer - *Philodina* sp.

x600

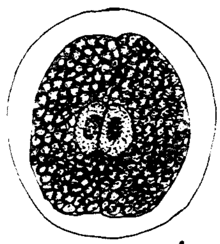


b. tropozoite.



c. syzgy.

x600



d. gametocyst.

x600

Fig.44.

when anchored by the foot or stretches forward to repeat the previous motion. As far as could be identified by the key given by Edmondson (1961), this rotifer was found to belong to the order Bdelloidea; family Philodinidae, and the genus Philodina, of which several species are recorded from New Zealand by Russell (1961).

The rotifer is commonly found on the legs, head, and prothorax especially, of the larva. It is very likely that in this position they cannore readily gain their food from the current of water drawn in by the undulatory movements of the abdomen. They are frequently seen affixed to the exoskeleton with the main body of the animal waving around in the water.

Despax (1951) says of the Trichopterous larvae that gregarines are frequently found in the gut; Gregarina anaboliae in Anabolia sororcula, G.limnophila in Limnophilus rhombicus and Diplocystis phryganeae in Phryganea spp. The Gregarine sp. in this case has not been identified (Fig.44 b). It is found fairly constantly in the gut cavity, the trophozoite lying free in the midgut where they are found in pairs forming syzgies (Fig.44 c). Gamocysts formed by the union of such a pair are seen in the midgut but more typically in the rectum, (Fig.46 d).

The exact nature of the relationship between these gregarines and the host is not clear. No analysis of a sample of larvae has been undertaken, but from observations made, it is known that a small number of the larvae do lack them. Since the larvae seem to survive equally well with or without this intestinal fauna, it may be deduced that both the gregarines and the rotifers are facultative commensals.

Finally there occurs frequently on various parts of the exoskeleton, chiefly the head and thorax, a fauna of various protozoa, among the more common of which are forms of Peritrichia (Ciliates), solitary and colonial stalked vorticellids. Some specimens, especially larvae that have been in aquaria, had their legs and head quite thick with these sessile ciliates.

## 2/4 ABBREVIATIONS

| | |
|---|---|
| a. | anus |
| ae. | aedeagus |
| ant.cl. | ante clypeus |
| an.pr. | anal processes |
| ant. | antenna |
| ant.lab.m. | anterior labral muscles |
| ap.sd. | aperture of silk duct |
| br. | bristle |
| c. | cardo |
| ch.pl. | chitinous plate |
| c.p. | coxopleurite |
| c.s. | coronal suture |
| cx. | coxa |
| d.c. | discoidal cell |
| d.pr. | dorsal process |
| e. | eye |
| ej.d. | ejaculatory duct |
| end.ph. | endophallus |
| f. | facet |
| fr.cl. | frontoclypeus |
| fe. | femur |
| f.s. | frontal suture |
| g. | gills |
| h. | hooks |
| h.pr. | hairlike processes |
| h.s. | haustellum |
| i.l. | inner lobe |
| inf.app. | inferior appendage |
| lab. | labrum |
| lab.p. | labial palp |
| lam. | lamella |
| lap. | lapett |
| lc. | lacinia |
| l.d.b. | lobe of dorsal branch |
| lig. | ligula |
| l.l. | lateral line |
| m. | mandible |
| m.b. | mandibular bristles |
| m.d.pl. | median dorsal process |
| me. | mentum |
| m.p. | maxillary palp |
| mxl. | maxillolabium |
| pap. | papilla |
| ph. | phallus |
| ph.ap. | phallic apodeme |
| ph.cr. | phallocrypt |
| ph.sc. | phallic sclerite |
| ph.tr.c. | phallotremal cavity |
| p.lab.m. | posterior labral muscles |
| p.o. | posterior occiput |
| pr. | truncate protruberance |
| pr.l. | pro leg |
| p.tt.p. | posterior tentorial pit |
| s.h. | swimming hairs |
| sk.r. | skeletal rod |
| s.m. | submentum |
| sp. | spine |
| st. | stipes |
| sub.g.pl. | sub genital plate |
| sup.app. | superior appendages |
| t. | tubercle |
| t.cl. | tarsal claw |
| ti. | tibia |
| tn. | trochantin |
| t.pr. | truncate protruberance |
| tr. | trochanter |
| ts. | tarsus |
| t.sp. | tarsal spine |
| v.b. | ventral branch |
| v.l.w. | lateral wing of ventral branch |
| viii | segment eight |
| ix | segment nine |

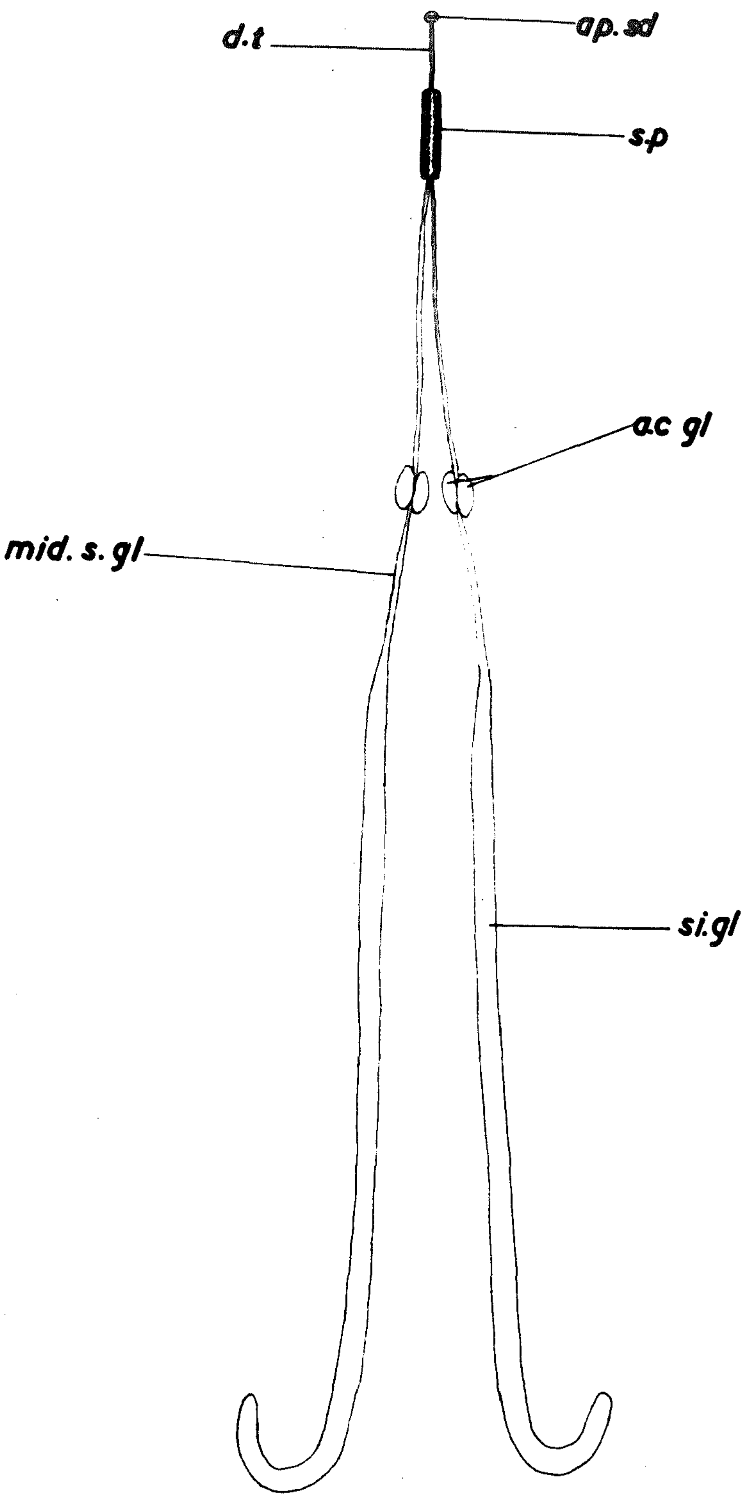



Fig.45. Diagrammatic representation of silk glands and associated structures.

# Chapter 5: FUNCTIONAL MORPHOLOGY.

## 5/1 The Larval Silk Glands.

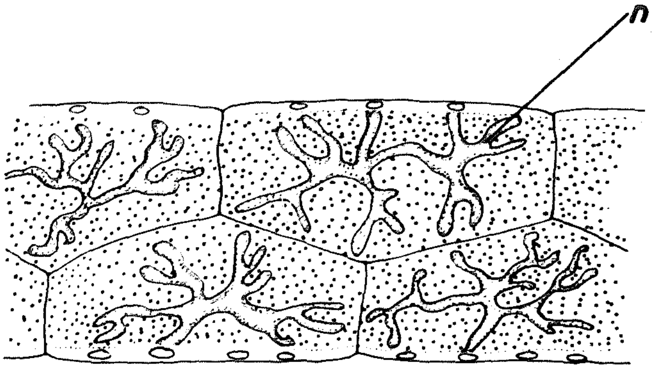
### i. Material and Methods.

Material to be sectioned whole was obtained fresh from ecdysis, since otherwise the head and thorax are too heavily chitinised for sectioning. The labium was also dissected out from freshly moulted larvae and sectioned. Material was fixed in Bouin and Carnoy, following the techniques outlined by Pantin (1948). Both fixatives gave satisfactory results. Material fixed in Carnoy resulted in well defined nuclei, while most of the histological detail was worked out from that fixed in Bouin. It was found that with Carnoy the silk filled the gland and had no structure that could be discerned. With Bouin, however, the silk fixed differentially into a solid mass about a third the diameter of the lumen of the gland and outside this filling the remainder of the lumen was a faintly striated much less dense layer. The centre core stained a dark purple-red while the outer ring of striated material was a pale blue when stained with Heidenhain's Azan. Sections were all cut at 10$\mu$ thickness.

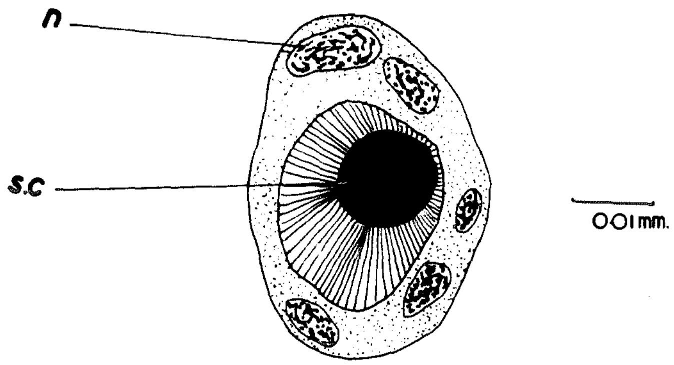
In order to determine the glandular part and the duct of the silk glands, transverse serial sections were cut. Also the path of the silk ducts in the labium and the exact relation of the accessory glands to the silk glands were determined.

### ii. The Anatomy and Histology of the Silk Glands and Accessory Glands.

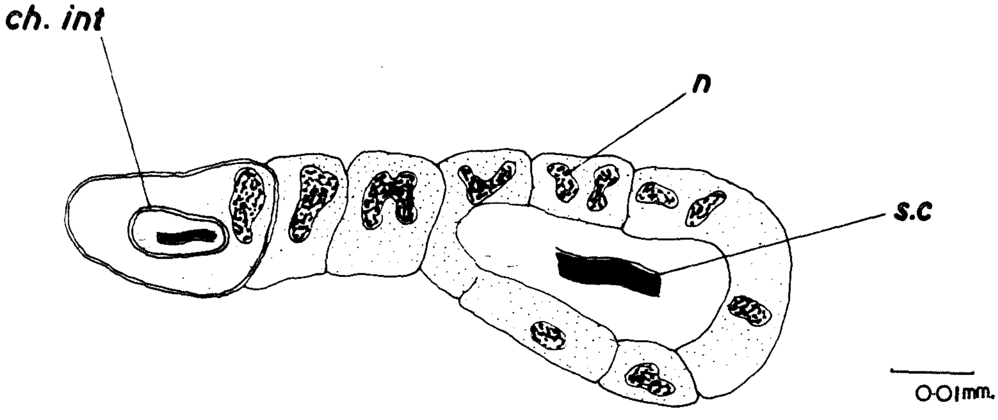
The silk glands or labial glands (Fig.45 si.gl.) in Triplectides obsoleta are well developed and extend from the labium anteriorly, and posteriorly as far as the 6th and sometimes 7th abdominal segment. Each gland then doubles back and ends in the previous segment tapering slightly




a. Diagrammatic of silk gland cells.




b. T.S. silk gland in metathorax.

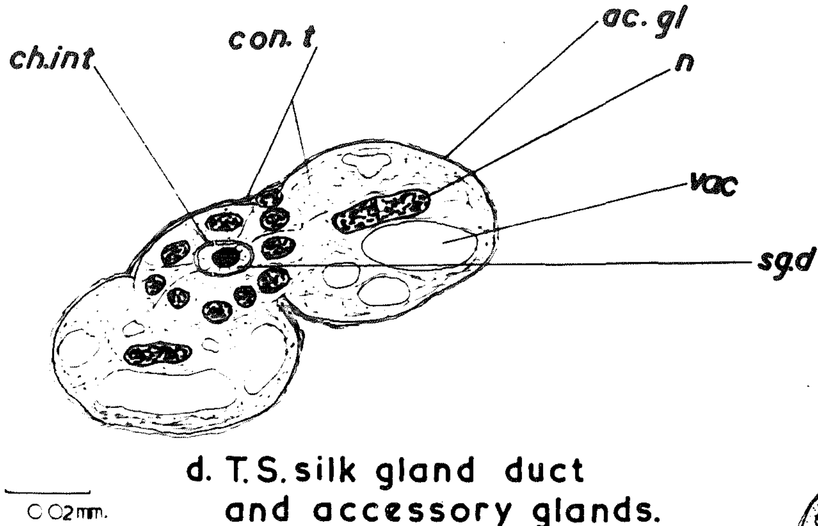



c. T.S. & L.S. of region where the silk gland passes into the silk gland duct.

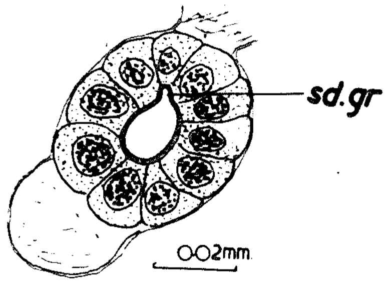
Fig.46.

to a rounded end. The glands are transparent structures and lie ventro lateral to the alimentary canal. Associated with these glands in the region of the mesothorax are accessory glands, double structures (Fig.45 ac.gl.); each half a small oval body on either side, and very closely applied to the walls of the silk gland. Just posterior to these accessory glands the silk glands narrow considerably and pass forward into the head as two very fine ducts (Fig.45 s.g.d.); these end in a single duct which opens at the tip of the labium.

The cells of the silk gland are large and elongate (Fig.46 a ), containing a granular cytoplasm with very irregular and often much branched nuclei. The gland is formed by a single layer of cells surrounding a central cavity filled with silk. The cells are of a uniform size throughout the posterior half of the gland which is fairly constant in size. Figure 46 b shows a transverse section of the gland in the metathorax and is typical also of the gland posterior to the thorax. Cell walls of the posterior portion of the gland have only been seen in longitudinal section (Fig.46 c). In the middle region of the gland (Fig.45 mid.s.gl.), usually in the prothorax, the cells become smaller and there is a marked decrease in the diamter of the gland. The nuclei are not so branched in these cells of the middle region and are smaller in size. This transition region between the posterior and mid-portion of the gland is shown in section in Figure 46 c. Here also can be seen the beginning of the true duct of the gland. This is indicated by the differentiation of the inner wall to form a double layered cuticular intima (Fig.46 c), which stains differently to the inner walls of the gland cells in the posterior part of

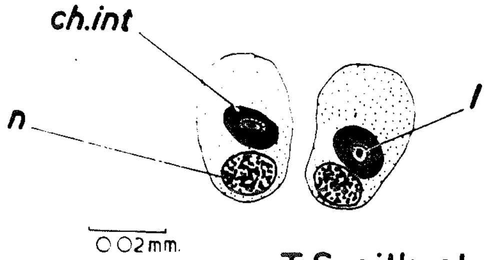



d. T. S. silk gland duct and accessory glands.



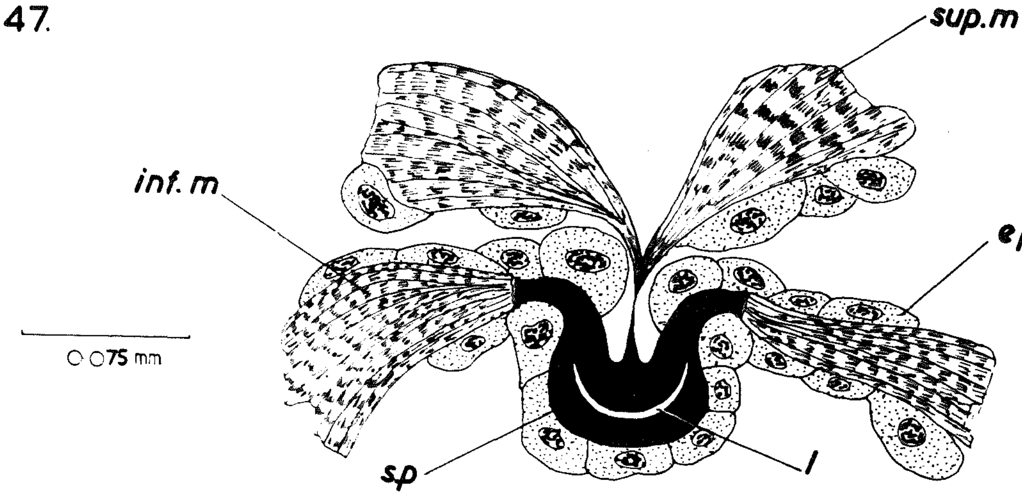

e. T. S. silk gland duct and anterior portion of accessory gland.

Fig.46.




a. T. S. silk gland ducts at base of labium.

Fig.47.




b. T. S. silk press.

the gland. Further forward, in the region of the accessory glands, the silk duct is more circular in outline, and the cells contain comparatively large nuclei. From seven to ten cells may be seen in cross section (Fig.46 d and e).

The accessory glands as far as could be seen from the sections, did not have any ducts. Where these accessory glands are in close contact with the walls of the silk duct, the cell boundary between them is very thin (Fig.46 d.). The products of the gland presumably difuse through these very fine cell walls. The cells of the accessory gland are poorly defined in cross section and there are usually only one, or at the most two large nuclei seen in a section, and these are more deeply staining than the nuclei of the cells of the duct. The gland is composed of reticular tissue and is considerably vacuolate (Fig.46 d vac.).

Immediately anterior to the region of the accessory glands the striated intima of the duct develops a groove (Fig.46 e s.d.gr.), the function of which is unknown. In the head region there is only one cell seen in cross section of the silk gland duct (Fig.47 a). The intima of the duct is very thick and the lumen narrow compared with the size of the duct. At the base of the labrum the ducts are still separate, but almost immediately their walls coalesce and finally as they enter the silk press (Fig.47 b s.p.), the lumen of the ducts fuse to form a single cavity. The lumen is flattened into a horseshoe shape (Fig.47 b l.) and is surrounded by the thick walled chitinous press, which is of a similar shape except that the ends are produced laterally to form two arms. Inserted on the ends of these arms are the inferior muscles (Fig.47 b inf.m.)

which arise ventrolaterally on the labium. A second pair of muscles are inserted in the centre of the dorsal wall of the press on a projection of chitin. These are the superior muscles (Fig.47 b sup.m.), and they arise dorsally on the labium. Large epithelial cells line the silk press ventrally, and these also surround the lateral arms and the base of the inferior and superior muscles (Fig.47 b ep.). In the larval Lepidoptera, Snodgrass (1935) says that both these sets of muscles apparently are dilators of the press lumen, the antagonistic force being the elasticity of the infolded dorsal wall of the organ.

The next portion, anterior to the press, is the directing tube. This consists of an outer layer of large epithelial cells, inside of which is a flat elongate chitinous intima (Fig.47c ch.int.). Just posterior to the tip of the labium, the directing tube is no longer flattened in form. The intima becomes very much thicker and the epithelial cells no longer form an outer layer. The directing tube is again curved upwards at the ends and the dorsal wall infolded protruding into the lumen, (Fig.47 d l.). Finally at the aperture the lumen is folded so that the bulge formed by the infolding of the dorsal wall is almost cut off (Fig.47 e ). The lumen is very narrow ventrally, while dorso laterally on either side, the lumen ends in a rounded cavity.

iii. <u>Functional Aspects of the Spinning Apparatus.</u>

The posterior part of the silk gland, from evidence of the glandular nature of the cells, is an actively secreting area; while the lumen of the gland acts as a reservoir for the silk substance. The smaller celled area in the middle region of the gland, may indicate that a substance of a different nature is secreted. Glasgow (1936) records that Jiro Machida found that in the silkworm the silk consists of two distinct substances, fibroin and sericin, the former being secreted at the posterior part of the gland and the latter in the middle region. He demonstrated this by a differential staining technique. Glasgow applied this to Hydropsyche but did not get the same results, the silk staining a uniform colour throughout. When it is considered that one of the constituents of the Lepidopteran silk sericin, is recorded as being water soluble, Roeder (1953) and Imms (1957), it is unlikely that it will also be a constituent of Trichopteran silk. Therefore it is not surprising that Glasgow obtained negative results with Machida's techniques. The exact chemical constituents of the Trichopteran silk has not been recorded, but from the differential staining of the silk, in Triplectides obsoleta, with Heidenhain's Azan, it would point to the fact, surely, of the existence of two constituents. This differential staining, however, was found throughout the posterior part of the gland, and results in the middle part were found to be similar. Anteriorly from this region the silk was not so well fixed and was difficult to define, with any certainty. From these observations it is deduced that the function of the smaller cells of the middle region of the gland therefore remains uncertain. It is obvious that before working out the exact functions of the different cells, it is necessary that the chemical constituents of the silk should be determined. Then it may be possible, by using differential staining techniques similar to those recorded by Glasgow and indicacted above, to obtain some results which would indicate more specifically the exact functions of the different regions of the silk gland.

An interesting feature of silk glands is the bilobed accessory gland of each duct, which is of doubtful function in Trichoptera. Imms (1957) says of Lepidoptera, "that the function of these glands often called

> Filippi's or Lyonnet's glands, is to secrete a substance of a liquid or viscid nature which enables the two threads to adhere within the spinneret and at the same time, facilitates the process of hardening."

In *T. obsoleta* larva these accessory glands are situated more posteriorly than in Lepidopteran larva, but they may still have a similar function. In all cases there is no experimental data, according to Roeder (1953) to support either of the above theories as to the possible function of Lyonnet's gland. Glasgow found that the silk glands of *Hydropsyche* had no such glands as seen here, and which are also apparently recorded by Gilson (1893) for some Trichoptera, for Glasgow says "there is no tuberosity such as Gilson thought represented Lyonnets gland". Instead he suggests "that the swelling of the epithelium of the silk duct in the

> region of the press is homologous with Lyonnet's gland in Lepidoptera, and that it should be noted that Gilson figures a similar investment of some of the muscles of the press, but describes it as 'amas de protoplasme non differerentié de la cellule musculaire'".

If Glasgow's suggestion holds good then how do these cells, which he calls Lyonnet's gland, secrete through the chitinous structure of the press? Here in *T. obsoleta* it would seem that the accessory glands on each duct are more likely to be homologous with Lyonnet's gland, while the cells round the silk press are merely large epithelial cells, the nuclei of which do not stain as deeply as the nuclei of the silk gland or accessory gland cells.

The function of the press, according to Packard (1909) is to mould the thread of the silk. The action of the muscles of the silk press (Fig.47 b) forces the silk through the directing tube. "When the muscles of the press are relaxed, the elasticity of the chitinous wall almost obliterates the lumen and holds the thread firmly as in a pair of pincers. Contraction of the muscles opens the press, permitting the passage of the thread, and by varying the openings of the press the larva can control the tension of the thread in its spinning" (Packard as quoted by Glasgow). This same function of the silk press is again recorded by Snodgrass (1935) and has been cited earlier in this section. It would appear from the arrangement of the muscles of the silk press and the form of the press itself, that since these features are similar to those of the silk spinning Lepidoptera larvae, the functions of the press in this species would be the same as indicated above.

From examination of the silk thread emitted finally from the aperture, it was noted that it appeared double. However, from the anatomy of the silk press and directing tube, which were most definitely single, it was decided that either the silk from each silk gland duct entering the press retained its own identity, or that the form of the lumen of the directing tube just prior to the aperture (Fig.47 e) fashions the silk into its final shape (Fig.47 f). The curving around of the lumen and its two rounded cavities at each end (Fig.47 e 1.) give the silk the appearance of being a double thread.

Glasgow compares the silk gland of Hydropsyche with those described by Gilson, which apparently agrees in most respects, except for some minor detail. Here in T. obsoleta there are also some differences compared with Hydropsyche. In Hydropsyche there is a thin dark intima throughout the gland, in T. obsoleta there is only an intima lining the duct. Glasgow records that this latter condition is also true in some of the species examined by Gilson. In Hydropsyche there is no small celled 'region conductrice' such as Gilson found in Limnophilus, but in T. obsoleta the small cells seen in Figure 46 e could well represent the 'region conductrice' of Gilson. Gilson evidently describes a tuberosity which he suggests represents Lyonnet's gland of Lepidoptera. This is not seen in Hydropsyche which has instead, according to Glasgow, large epithelial cells surrounding the silk press and associated muscles. In T. obsoleta there are found both the tuberosities which according to the descriptions given by Packard (1909), are more likely to represent Lyonnet's gland; and also the large epithelial cells associated with the press, which from observations would not seem in this species to have any glandular function.

## 3/2 Comparative Functional Morphology of the Larval, Pupal and Adult Alimentary Canal.

### i. Introduction.

Because of the nature of the food, the functions of the larval and adult alimentary canal are very different: it was therefore decided that some interesting comparisons could be made by examining the anatomy of the alimentary canal. The pupal alimentary canal was briefly investigated, as this showed a transition stage between the larval and adult condition. As seen in the section dealing with food and feeding of the larva, the alimentary canal of this stage has to be so adapted, to cope with bulky plant material. On the other hand, the adult takes up liquid, but not solid food material.

Glasgow (1936) has dealt in some detail with the larval structure of the alimentary canal of Hydropsyche and compares it with some other Trichopterous larvae. He also examined the structure of the adult alimentary canal of the male of this species. Deoras (1944) gave descriptions of the gut in eight species from six families and these were shown to have the same general form as that which will be seen below.

### ii. Material and Methods.

Larval material fresh from ecdysis was fixed in Bouin and Carnoy, and sectioned whole for details of the alimentary canal. The alimentary canal was also dissected out of feeding larvae and sectioned longitudinally. Pupal material was fixed in Weaver and Thomas fluid specially recommended for material of this nature to be dissected (Weaver and Thomas 1956). Pupal material was available too late for further detail other than that

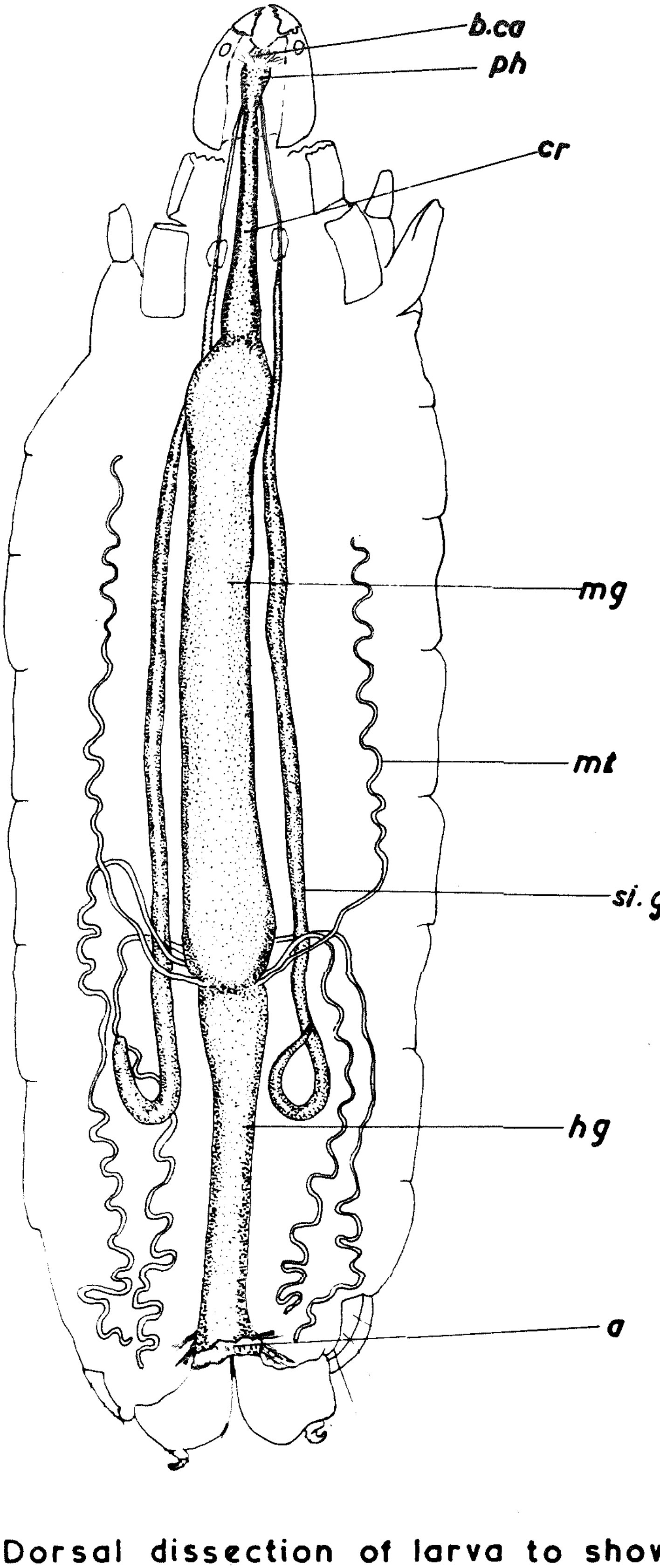



Fig.48. Dorsal dissection of larva to show alimentary canal.

which could be obtained from gross dissections. The adult material was fixed in Carnoy for from three to eight hours. This was used chiefly because it is a good fixative for insect material, being a very quick penetrant. Only teneral adults were used for sectioning, so that the chitin was still soft enough for cutting in paraffin wax with a melting point of 52°C. Sections were cut from whole specimens as it was found too difficult to dissect out the alimentary canal from fixed material, without damaging the tissues. At the time when this investigation was done no fresh adult material was available, and that which was used had been fixed for several months, and stored in 70% alcohol. All sections were cut at 10$\mu$. Sections were stained in Chlorarol Black and Delafields Haematoxylin, both of which resulted in well stained nuclei. It was decided however that in order to show up different tissues, Heidenhains Azan was used. This stain was used according to Pantin (1948) and gave good, clear results.

iii. The Larval Alimentary Canal.

The most prominent feature of the internal anatomy of the larva is the large tubular alimentary canal, the midgut filling most of the abdominal segments. A short buccal cavity (Fig.48 b.ca.) leads into the pharynx (Fig.48 ph.) which extends to the posterior region of the head, where the crop begins (Fig.48 cr.). This is a little wider than the pharynx and may become distended posteriorly according to the amount of food contained. This is usually a liquid as most of the solid material passes quickly into the midgut. The epithelium of the foregut is folded longitudinally and there is a chitinous intima throughout.

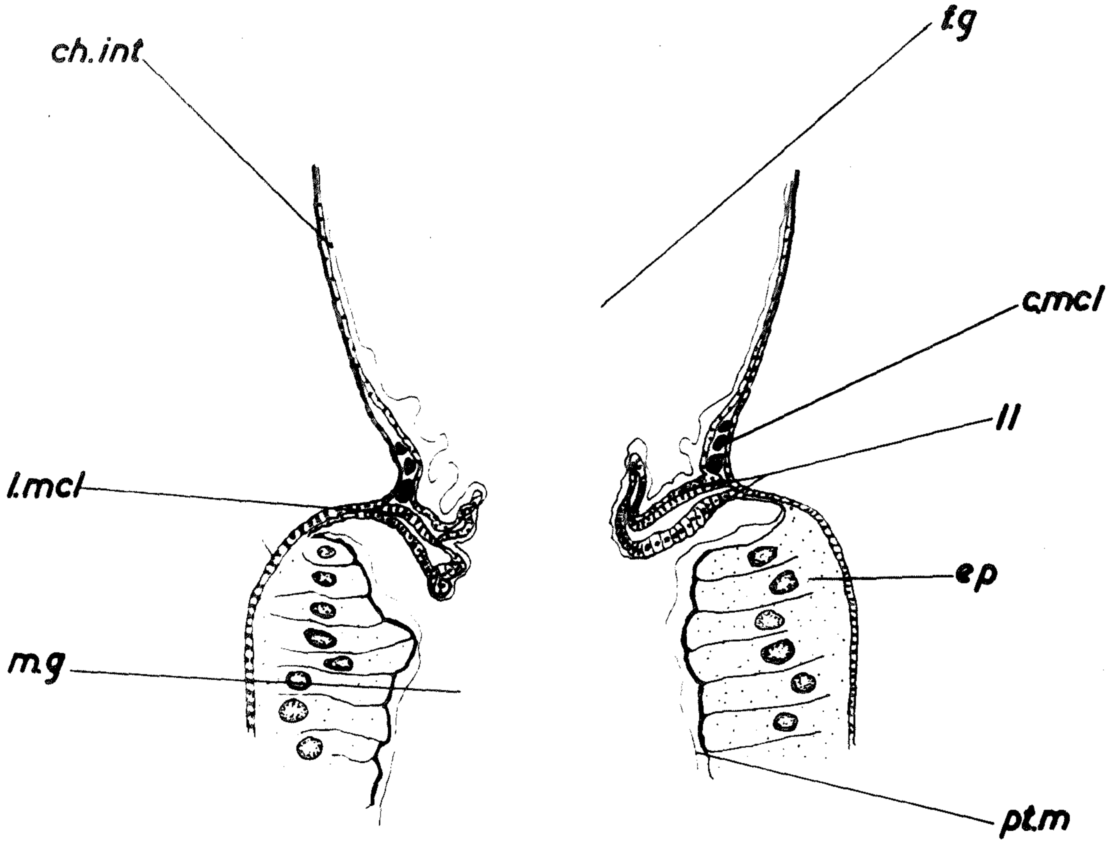



Fig. 49.a. L.S. cardiac sphincter.

There is a constriction between the foregut and midgut brought about by a thin band of circular muscles. There is no well developed oesophageal valve as recorded by Glasgow for Hydropsyche. Instead the folding of the epithelium at the posterior end of the crop is increased and there is a very weakly developed infolding of the foregut into the midgut. The two sides of this lamella (Fig.49 a ll.) are not apposed and between the two sides is a longitudinal muscle (Fig.49 a l.mcl.), which, when contracted compresses the lamella. The chitinous intima of the foregut is present on both sides of the lamella posterior to which there is a crypt separating it from the beginning of the midgut cells. The relationship of this area is shown in Fig.49 a. It is concluded that the opening and closing of this area is governed by the circular muscles forming the cardiac sphincter (Wigglesworth 1953), the infolding of the foregut epithelium being too small to act as a valve as seen in Hydropsyche. However it retains one of the main functions of an oesophageal invagination, this being to ensure that the contents enter within the peritrophic membrane.

The midgut is an elongated wide tube extending from the first to approximately the sixth abdominal segment. In the feeding larva it becomes considerably distended and is packed from end to end with plant fibres and other food material mentioned earlier. It is this region of the alimentary canal that digestive processes go on. This is well indicated by the nature of the epithelium which is composed of tall columnar cells with a striated border. The epithelium is fairly uniform throughout the midgut. From longitudinal sections of actively feeding larvae it was found that the more posterior epithelial cells had their free

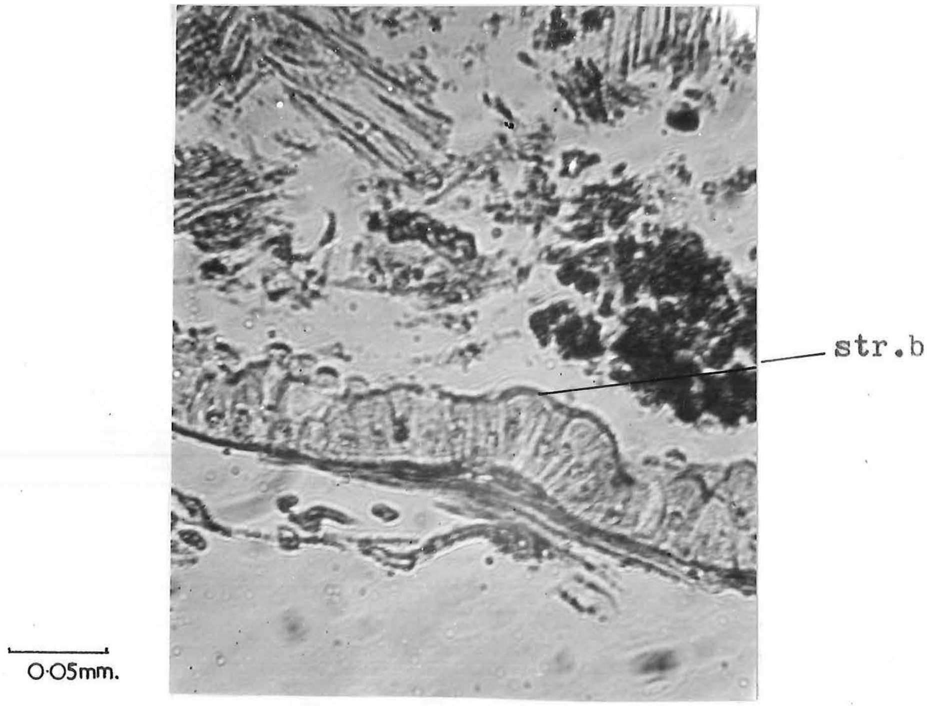



Fig.49b Epithelial cells of anterior midgut

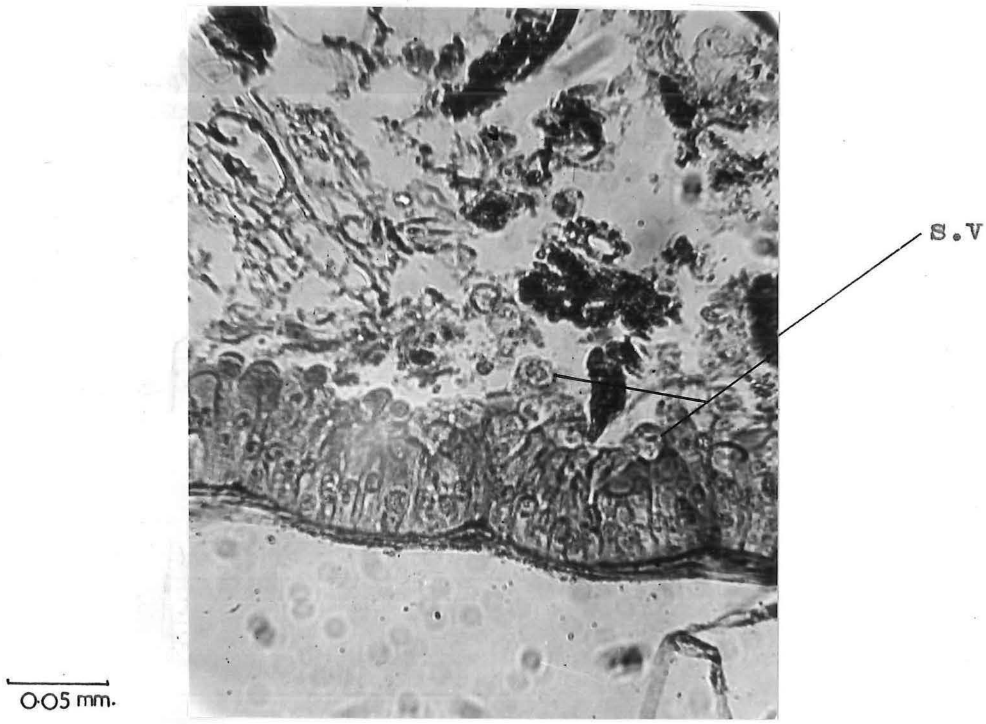



Fig.49c Epithelial cells of posterior midgut.

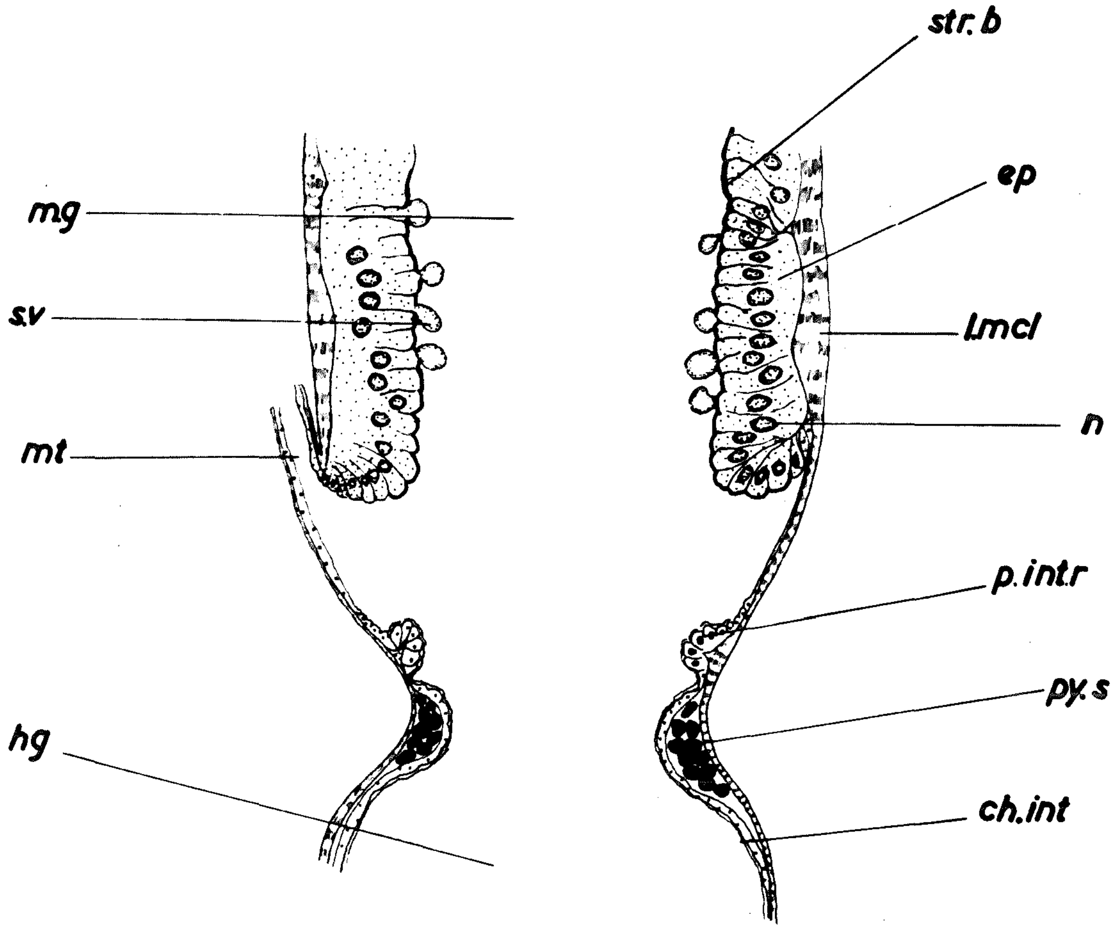
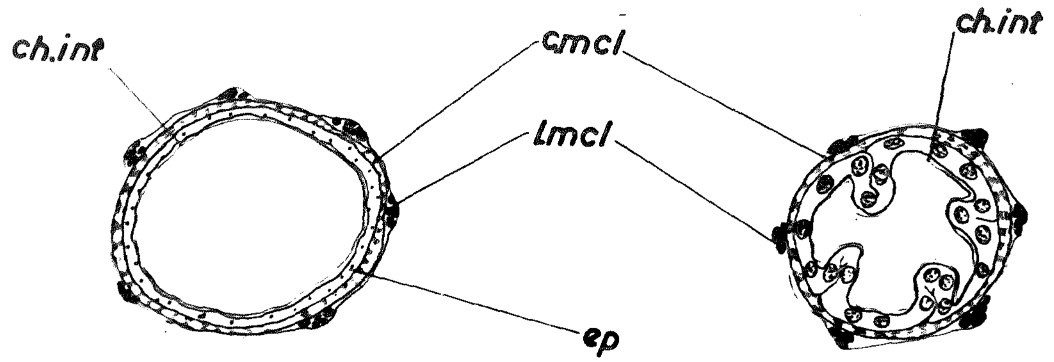



Fig.50. a. L.S. pyloric sphincter and entrance of a malpighian tubule.

ends produced to form secretory vesicles (Fig.49 c s.v.) which were freed into the lumen. In the anterior region of the midgut, the epithelial cells seemed to lack these vesicles and the striated border was unbroken (Fig.49 b str.b.). It is likely that the anterior region is mainly an absorptive area, while posteriorly the cells are mainly secretory. A well developed peritrophic membrane lines the lumen of the midgut. There is an outer layer of longitudinal muscles with a less well developed inner layer of circular ones.

The six malpighian tubules (Fig.48 mt.) indicate the junction between the midgut and hindgut. Four of these are attached close together, a pair on either side, dorsolateral in position. The more dorsally situated one from each side passes forward to the anterior region of the abdomen. The second pair together with the third pair which is situated slightly apart and ventrolaterally pass back to the posterior region of the abdomen. The base of each tube becomes slightly dilated and opens into a cleft formed by the termination of the midgut cells anteriorly and a ring of small cells posteriorly which marks the beginning of the hind gut (Fig.50 a p.int.r.), and for the sake of convenience it is called the posterior intestinal ring. From just in front of this ring of cells the chitinous intima begins and continues posteriorly throughout the hind gut.

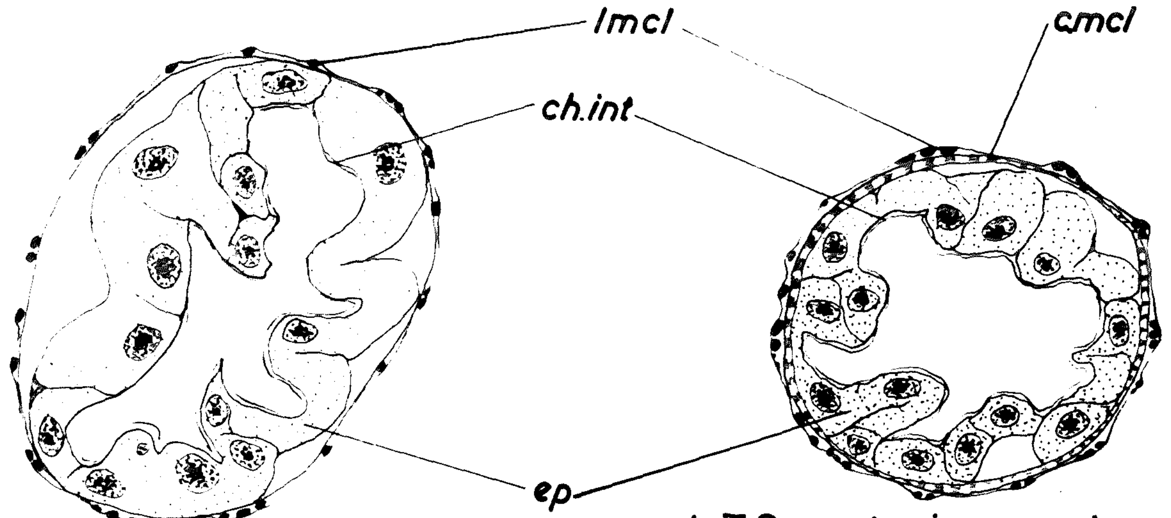
The hind gut (Fig.48 ) which may be divided into the anterior intestine and rectum, begins with the posterior intestinal ring. Situated a little posteriorly is the pyloric sphincter (Fig.50 a py.s.), the circular muscles of which are well developed. The first portion of the




b.1. T.S. ilium.
x150

b.2. T.S.colon
x150

Fig.50




a. T.S.anterior rectum.

b.T.S.posterior rectum.

Fig.51.

anterior intestine (Fig.48 ant.int.) is the ilium, which resembles a thin walled sac, the epithelium is thin and composed of small flattened cells. Posteriorly the ilium narrows and passes into the colon, the epithelium of which becomes folded longitudinally and the cells are slightly larger than those of the ilium. The colon (Fig.50 a 2.) forms a slightly narrower tube than the ilium (Fig.50 b 1.), and both areas have an inner layer of circular muscles and an outer layer of longitudinal muscles (c.mcl. and l.mcl.). The colon tapers posteriorly and there occurs a slight constriction between this latter area and the rectum (Fig.48 r.). This constriction is caused by a sphincter, which, when the circular muscles contract, brings the longitudinal folds of the epithelium in close apposition, and thus the passage of material is occluded. The rectum (fig. 48 r.) is much wider than the colon and the musculature is very thin, the circular muscles have not been identified with certainty except in the posterior rectum. The epithelium of the rectum is thrown into longitudinal folds and the cells are large and cubical in shape in the anterior rectum (Fig.51 a). The posterior rectum has a thin layer of circular muscles, the epithelial cells only slightly smaller than in the anterior rectum and the epithelium itself is still folded longitudinally (Fig.51 b). There is no anal sphincter; the anus opens more ventrally than posteriorly through a slit formed by the apposition of the prolegs.

iv. The Pupal Alimentary Canal.

The pupal alimentary canal (Fig.52) has only been examined from gross dissection. From Figure 51 it is evident that already the alimentary

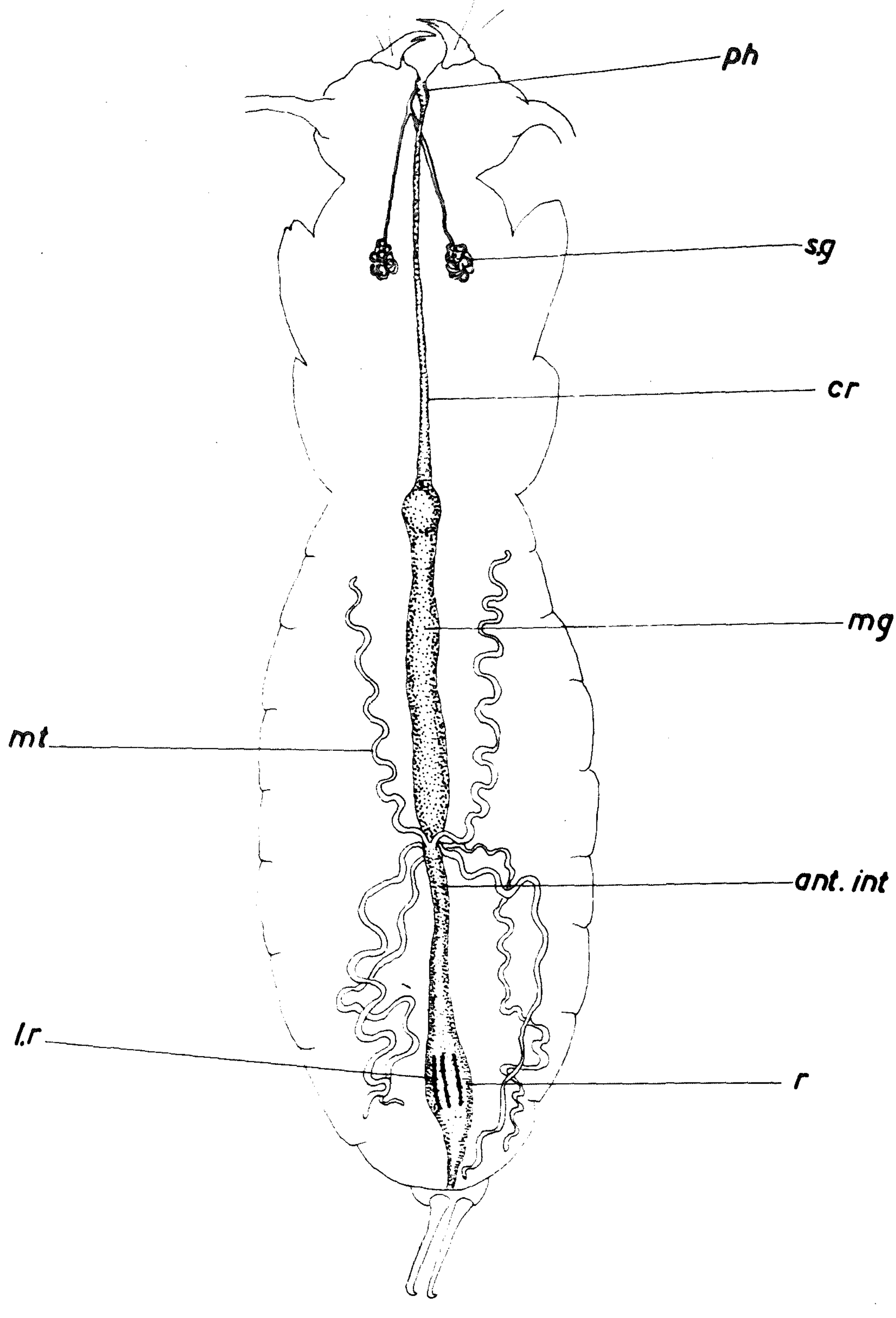



Fig.52. Dorsal dissection of pupal alimentary canal.

canal is beginning to resemble the form of that of the adult (Fig.53) although it is still a straight tube, as seen in the larva (Fig.48). The silk glands have been transformed into salivary glands (Fig.52 s.g.) which are two narrow white tubes extending from the haustellum where there is a single duct. This then divides into two, and ends in the mseothorax in several convolutions.

There is a short pharynx (Fig.52 ph.) which leads posteriorly to the crop which is a narrow semitransparent tube. In the region of the first abdominal segment the crop widens and enters the midgut (Fig.52 mg) which is less than half the size of the larval midgut. Posteriorly in segment five the junction of the midgut and hind gut is indicated by the opening of the malpighian tubules (Fig.52 mt.). They are similar in form and position as those of the larva. The anterior intestine (Fig.52 ant.int.) extends from segment five to segment eight where the rectum begins (Fig.52 r.). The rectum is divided into an anterior and a posterior part. The anterior rectum has six longitudinal ridges (Fig.52 l.r.) which probably indicate the future site of the adult rectal pads. The posterior rectum consists of a short narrow tube which leads to the anus.

The alimentary canal as described here, is that of the early pupa. There are further transition stages as seen between the 6th instar and the pupal stage and between this early pupa and the late pupal stage, when the alimentary canal closely resembles that of the adult.

The early pupal alimentary canal is still a straight tube and differs mainly from the larval alimentary canal in the lack of food content and

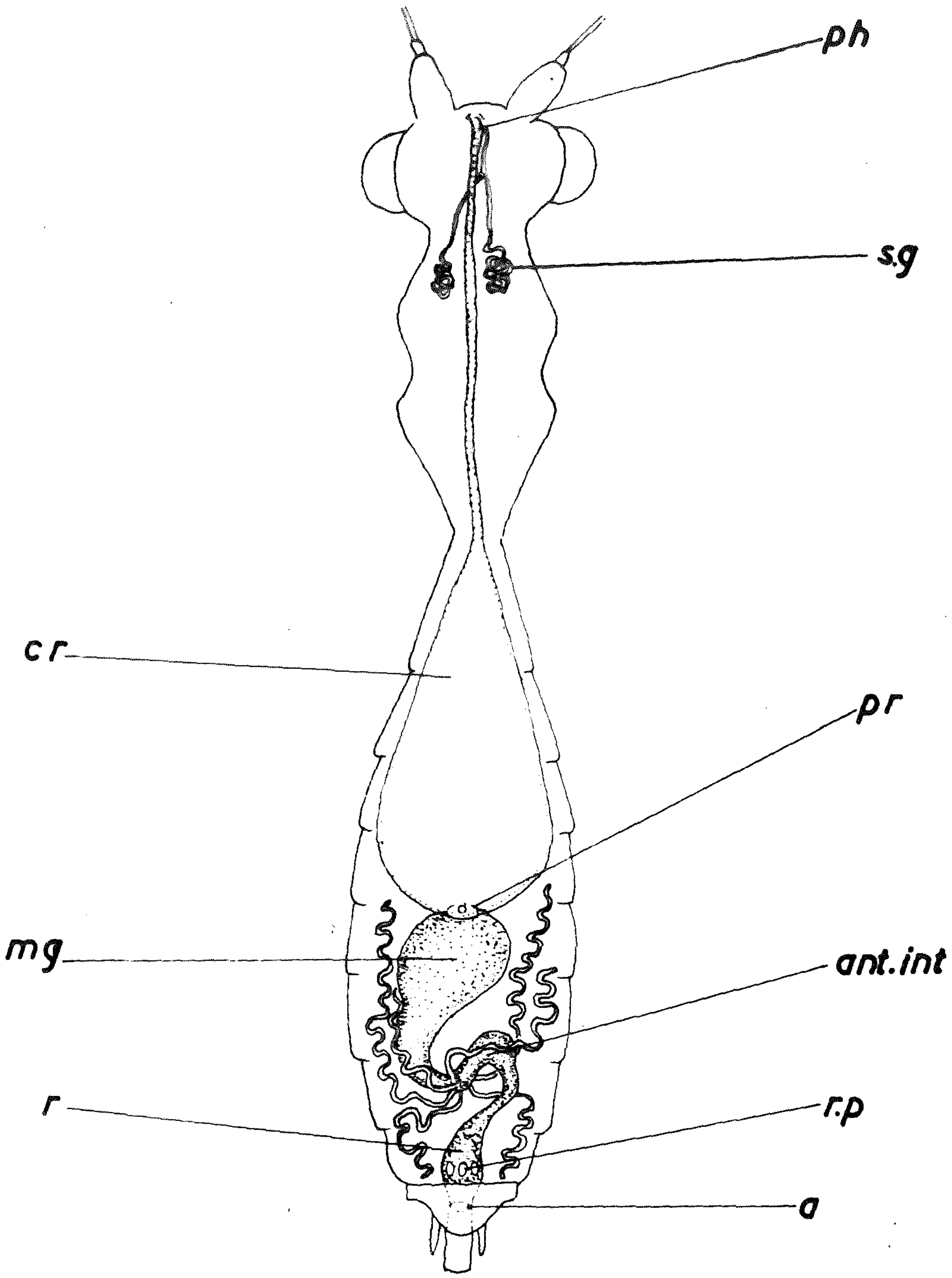



Fig.53. Dorsal dissection of adult alimentary canal (male).

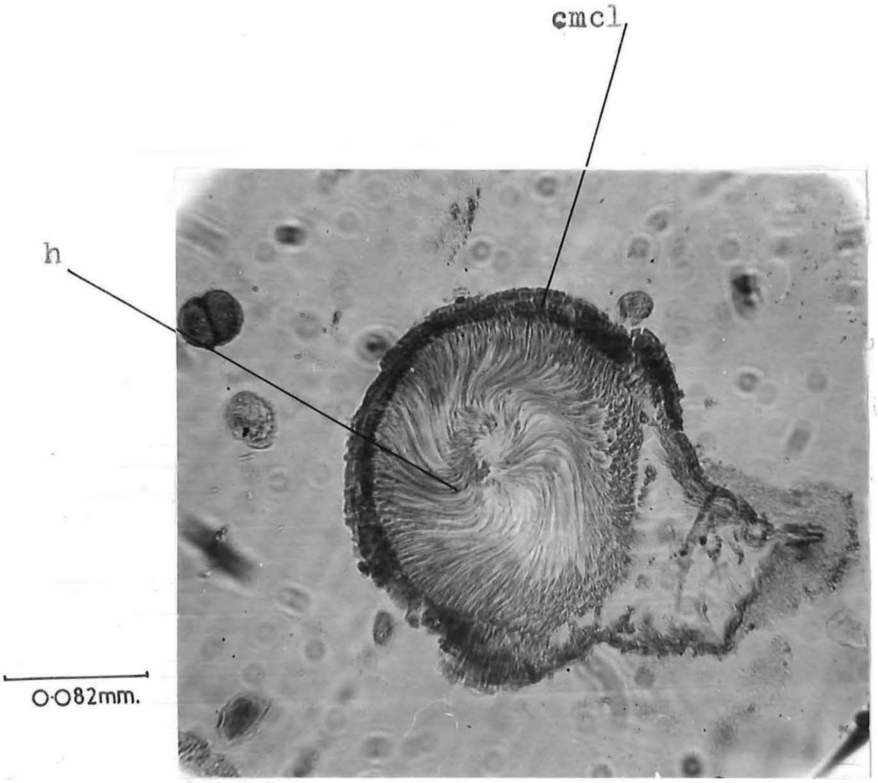


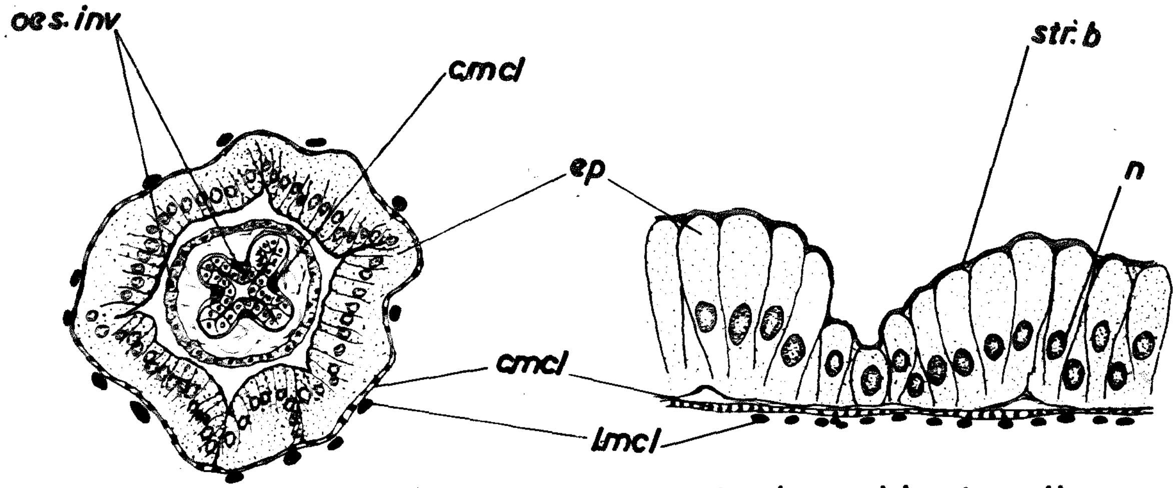

Fig.54a T.S. Proventriculus.

anatomically in the marked decrease in diamter of all parts of the canal. The transformation of the silk glands into salivary glands is another feature of the pupal stage.

v. The Adult Alimentary Canal.

The mouth opening is at the base of the haustellum and leads into a short pharynx (Fig.53 ph.), alongside of which are seen the salivary glands, which are similar in form to those seen in the pupal stage (Fig.53 s.g.). The pharynx quickly gives way to the crop which has thin smooth walls, and continues posteriorly through the thorax as a fine tube (Fig.53 cr.). In the first abdominal segment the crop becomes distended with air so that it occupies most of segment one to segment four. The wall of the crop is composed of a feebly developed musculature of an outer circular muscle layer and an inner longitudinal muscle layer. The epithelial lining is very fine and is covered by a very thin chitinous intima.

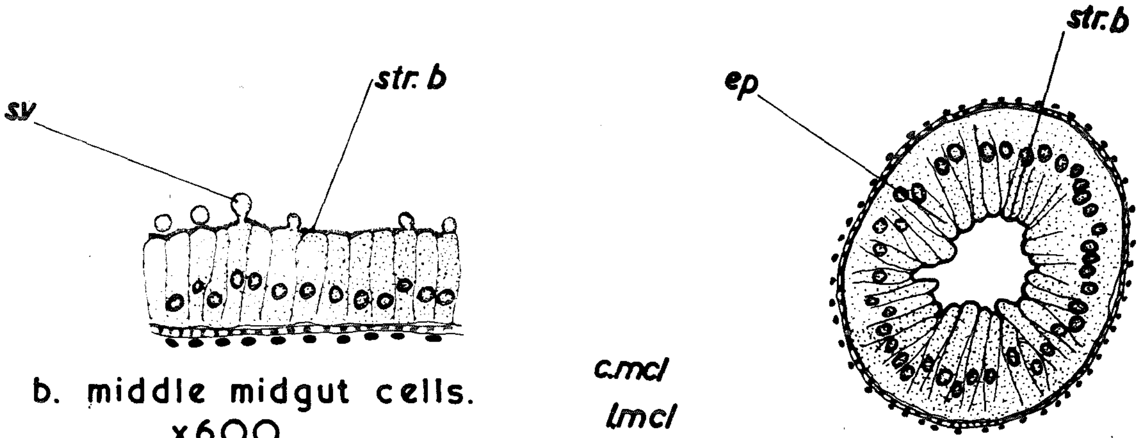
The fore gut and midgut are separated by a well developed muscular proventriculus (Fig.53 pr.). This is divided into an anterior and posterior part. The anterior portion consists of a very thick outer layer of circular muscles and the epithelium consists of long thin cells, the free ends of which are produced into long hairlike processes (Fig.54a h.). These processes project into the lumen in a wave-like fashion as shown in Figure 54a. The posterior part of the proventriculus forms the oesophageal invagination. The epithelium of the foregut is produced into four major longitudinal folds, these project into the midgut (Fig.54 b oes.inv.). The outer wall of the invagination forms a straight cylinder, and the space between the two sides of the invagination is occupied by




b. T.S. midgut and oesophageal invagination. x150

a. anterior midgut cells. x600

Fig.54.




b. middle midgut cells. x600

c. posterior midgut cells. x600

Fig.55.

connective tissue and a continuation of the circular muscles of the fore gut, beneath the folded epithelium (c.mcl.). This proventricular mechanism ensures that the passage between the fore and midgut may be regulated, merely acting as a sphincter, but a much more elaborate one than that seen in the larva.

The midgut (Fig.53 m.g.) is bulbous anteriorly while more posteriorly it narrows before passing into the hindgut. The midgut is folded so that the posterior end is lateral to and slightly above the anterior portion. The epithelial cells of the midgut exhibit three morphological forms. Anteriorly the epithelium is thick, slightly folded, and the cells are mostly tall and rounded at the ends with a narrow neck, some shorter cells also occur (Fig.55 a ep.). Where the midgut begins to narrow (Fig.53 m.g.) the epithelium is straight, the cells being smaller and more regular in outline than in the anterior region. This second area of smaller cells is characterised by the occurrence of secretory vesicles (Fig.55 b s.v.) indicating an actively secreting area of epithelium. In the posterior region of the midgut the epithelium is thick and the cells very regular in outline (Fig.55 c ep.). Throughout the midgut the epithelial cells have a striated border (str.b.) and both an inner layer of circular muscles and an outer layer of longitudinal muscles (l.mcl. and c.mcl.) are present.

Externally the midgut is seen to narrow towards the junction with the hindgut (Fig.53 h.g.). Here the malpighian tubules are located. These have the same general form and disposition as in the pupal and larval stages. In longitudinal section (Fig.56 a) it is seen that the pyloric

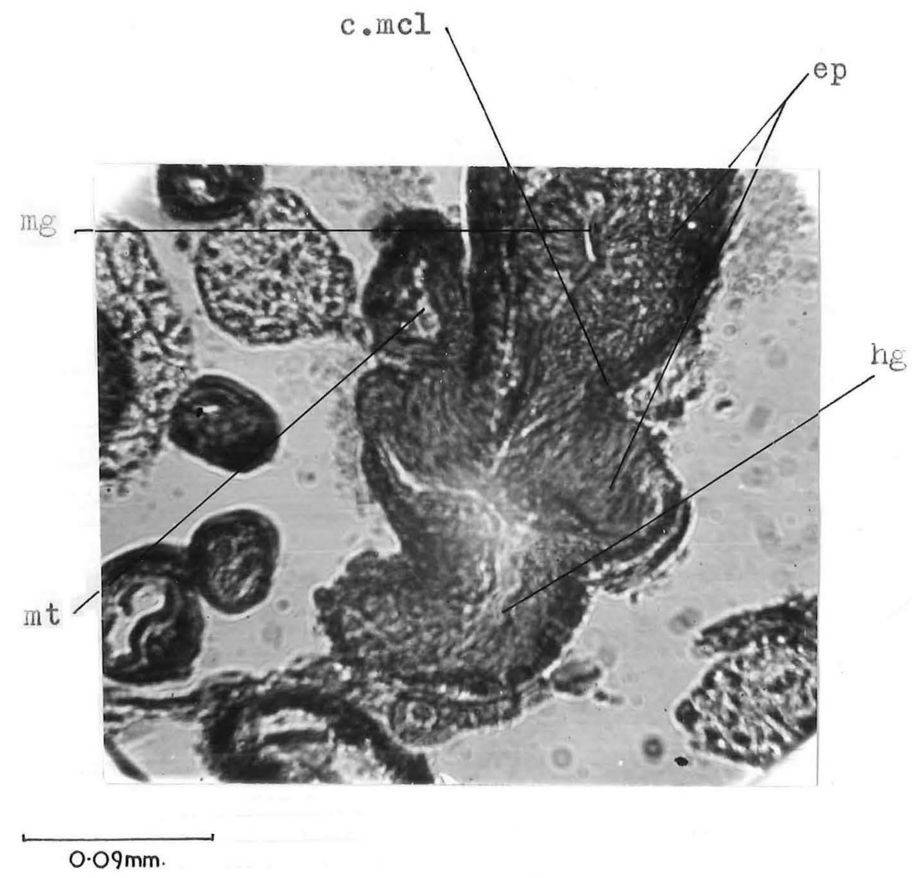
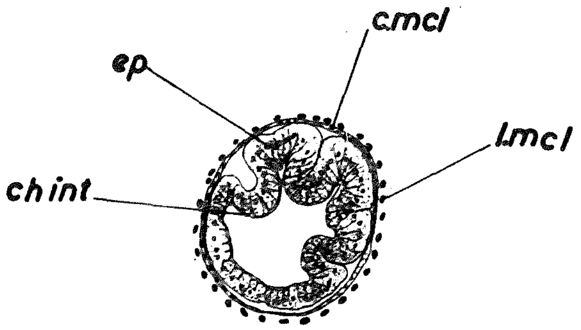
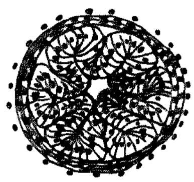



Fig.56a L.S. Pyloric valve

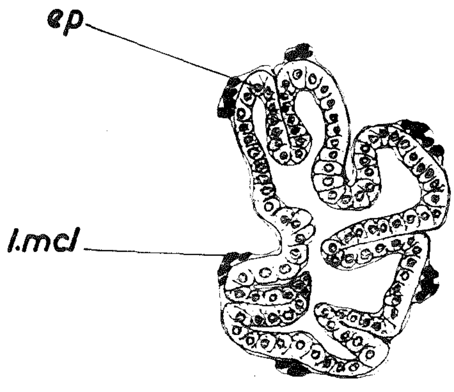



b. T.S. anterior ilium.
x600

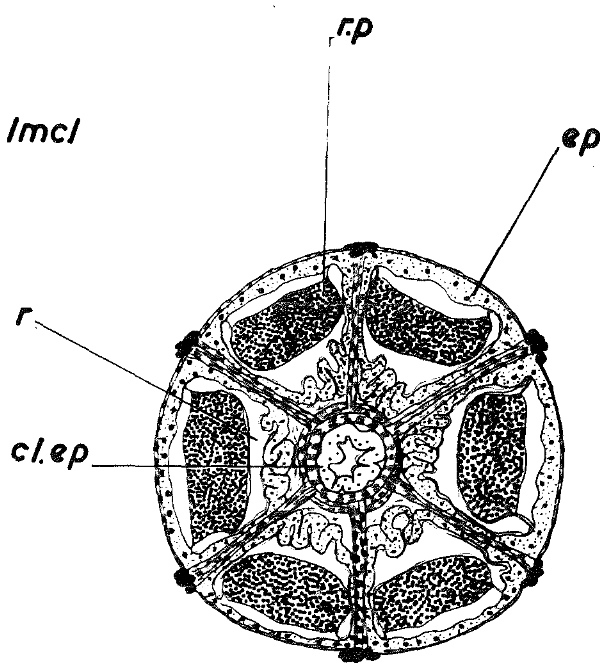


c. T.S. iliocolonic
sphincter.
x600

Fig.56.




a. T.S. colon.
x600

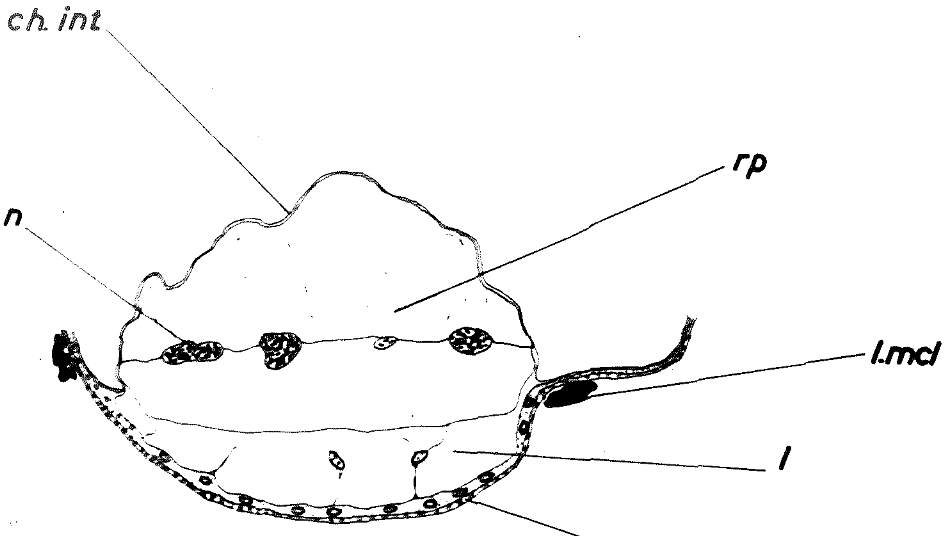



b. semidiagrammatic
representation of rectal
valve in T.S.

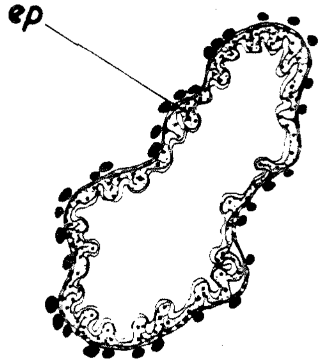
Fig.57.

valve is fairly well developed. The epithelial cells are long and so arranged that they form a thick ring (Fig.56 a ep.), which fills a greater part of the lumen. Laterally and lying parallel with the gut wall are the bases of the malpighian tubules, and these enter the lumen parallel with the wall of the midgut (mg.). The circular muscles (c.mcl.) of the midgut just before the pyloric valve are very thick and serve as a pyloric sphincter.

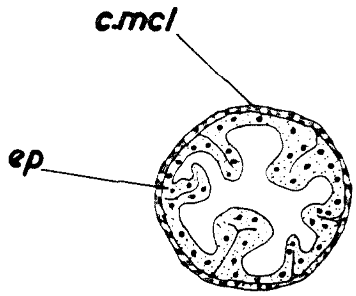
The anterior intestine (Fig.53 ant.int.) is curved round on itself and leads posteriorly into the rectum (Fig.53 r.). After the entrance of the malpighian tubules the epithelium is folded into six longitudinal ridges, and both an inner layer of circular and an outer longitudinal muscle layer are evident (Fig.56 b ep,c.mcl. and l.mcl.). This portion of the anterior intestine is the ilium which narrows considerably from before backwards, while the folds of the epithelium become closely apposed, ending in the formation of a sphincter (Fig.56 c sph.), similar to that seen in the larval hindgut. In both instances this sphincter divides the ilium from the posterior colon. This is wider than the ilium and consists of less regularly compacted epithelium (Fig.57 a ep.), and only the longitudinal muscles have been seen. Posteriorly the colonic epithelium becomes again compacted and thrown into six longitudinal folds. The circular muscle layer is thickened here and there are six bands of longitudinal muscles. This posterior end of the colon is invaginated into the rectum to form the rectal valve (Fig.57 b). The outer layer of the valve is histologically rectal epithelium and this is divided by the six longitudinal muscle blocks which continue on into the rectum lying




c. rectal pad.
x600




d. T.S. anterior rectum.
x600




e. T.S. posterior rectum.
x600

Fig. 57.

between the rectal pads. The lumen of the anterior rectum is occupied by the development of the six rectal pads on the periphery, and in the centre for a short distance, the rectal valve. The wall of the anterior rectum is composed of six outer longitudinal muscle blocks (Fig.57 c lmcl.) and an inner circular muscle layer (c.mcl.). Between the rectal pads the epithelium is thin and covered with a chitinous intima, which is continuous also over the rectal pads. Each rectal pad (Fig.57 c r.p.) is composed of a lower thin layer of epithelium continuous with that of the rectal wall and is followed by an extensive lumen (l.). The actual pad is composed of an outer deeply staining area in which no cellular detail can be determined. The outer layer is very finely granulated and no cell detail can be seen. Between the two layers may occur from two to four large nuclei (n.). The inner boundary is of course covered by the chitinous intima (ch.int.). Behind the rectal pads the anterior rectum consists of an irregularly folded epithelium (Fig.57 d ep.) and the longitudinal muscles are dispersed to form an almost continuous muscular wall. An inner circular muscle layer is also present.

The posterior rectum is a short narrow tube, slightly longer in the female. The epithelium is closely folded (Fig.57 e ep.) and there is a well developed layer of circular muscles (c.mcl.). In the ninth segment of the female the dorsal wall of the posterior rectum gives way to a very fine epithelium and chitinous intima continuous with the chitin of the exoskeleton. The ventral wall of epithelium remains folded and is continuous posteriorly with the soft fold of tissue which lies between the anus and the gonopore (Fig.58 c a., gp.). The two openings lie at the

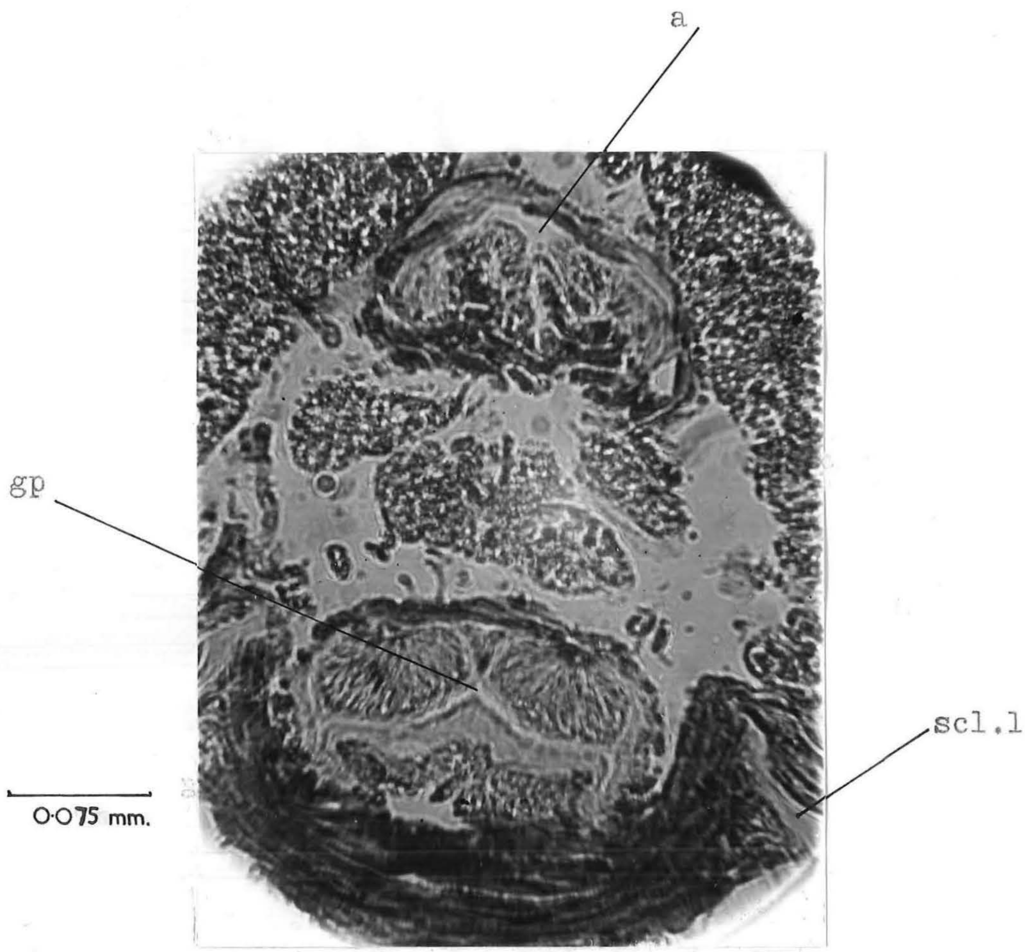



Fig.58 T.S. through segment nine to show relative position of anus and gonopore.

posterior end of segment nine between the bases of the sclerotised lamellae (scl.l.). A transverse section through this area shows this arrangement clearly (Fig.58). In the male the posterior rectum finally becomes thin walled and passes into the anus which opens ventrally on segment ten at the base of the dorsal process.

vi. Summary and Conclusions.

It is evident that the nature of the food of the larva requires an increased area of digestion and so we see the development of the spacious midgut, which is closed and opened anteriorly and posteriorly by sphincters. The crop in the larva would appear to be a reservoir for a coloured liquid which is frequently observed on dissection and is probably digestive fluid and micro-organisms which have come forward from the midgut. The crop of the adult on the other hand soon after emer ence becomes distended with air, the exact significance of which in this species is doubtful. It may however serve to lower the specific gravity of the insect, as Hunter suggests (recorded by Wigglesworth 1953), and so assisting in flight.

Compared with the larva the adult has a well developed oesophageal invagination as well as a proventriculus which control the entrance to the midgut. This is usually filled with liquid. In sections the fluid contents stain a dense bright blue with Heidenhains Azan. Posteriorly there is a pyloric sphincter with also a pyloric valve; this latter structure is again not present in the larva. It is suggested that a liquid diet entails the development of these invaginations to form valves in order that a more efficient control is given to the movement of the liquid contents. The development of the proventricular mechanism may also be involved with controlling the amount of air in the crop, and the hair-like processes of the proventriculus preventing the passage of air posteriorly into the midgut. The food contents of the larval midgut is contained within a very well developed peritrophic membrane while that of the adult has not been seen with a magnification of up to x600.

A further comparison is made between the anterior rectum of the larva and adult. In the larva the epithelium is composed of large cubical cells while in the adult there is seen the development of rectal pads. This is interesting since Wigglesworth points out that these rectal organs play an important part in the process of absorbing water, so it is that the aerial form of this species is found to have rectal pads. Wigglesworth also records the fact that the rectal pads of *Limnephilus* has been shown to take up chloride. Finally the large epithelial cells of the larval anterior rectum may represent a transition stage between the uniform epithelium and the rectal pads proper. Consequently these cells may also possess the functions of the rectal pads which may also entail the reabsorption of nutrients as shown for some species by Wigglesworth. Snodgrass (1935) states that the glandular function of these rectal organs has not been demonstrated.

In the larva and adult hindgut there are sphincters between the ilium and colon and colon and rectum. In the adult there is also however a rectal valve present.

In conclusion it may be said that the passage of the bulky food of the larva requires the presence of efficient sphincters. The liquid contents of the adult alimentary canal however, besides sphincters, requires the development of a proventriculus and also invaginations to form valves.

## 3/3 The Male and Female Reproductive Systems.

### i. Introduction.

Concerning the anatomy of the male and female reproductive systems, there are but few records in the literature. Glasgow (1936) gives a description of the general form of the male reproductive system of *Hydropsyche* and Deoras (1944) gives an account of the internal morphology, including the reproductive systems of eight species of adult Trichoptera belonging to six different families. Two of the species described belong to the family Leptoceridae and are useful for comparison with the reproductive systems of *Triplectides obsoleta*. As in Glasgow's work, Deoras only describes the general form of the systems and no anatomical detail of the various organs is given. An earlier work however by Dodson (1935) describes the development of the female genital ducts in Trichoptera, and the main part of the work is preceeded by a brief description of the form of the female reproductive system. Finally Despax (1951) gives a short account of the male reproductive system and mentions in particular some of the variation seen in the male reproductive organs.

### ii. Material and Methods.

The adult 'flies' were fixed in Carnoy. Specimens to be used for sectioning were left for from 12 to 16 hours in the fixative. Investigations were made by gross dissections of the fixed material using a binocular microscope. The results from these were confirmed by the examination of fresh material and by cutting serial sections. The stains used were Chlorazol Black, which gave good results for the development of oocytes within the ovarioles, but the more satisfactory stain used was

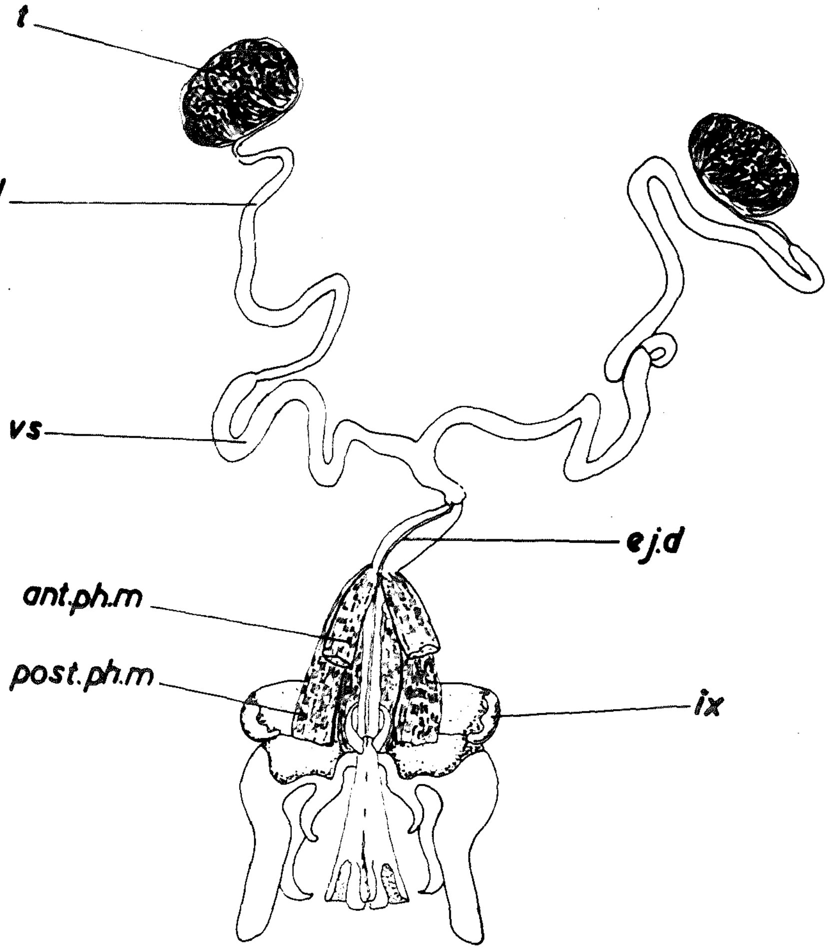



Fig. 59. Dorsal dissection of male reproductive system.

Heidenhains Azan. It was from sections stained in this that nearly all the anatomical detail was obtained.

iii. The Male Reproductive System.

The reproductive system in the male of T. obsoleta (Fig.59) is represented by a pair of testes followed by a series of ducts which finally meet to form the single ejaculatory duct. The testes (Fig.59 t.) are separate and situated laterally in the fifth abdominal segment. They are rounded white organs surrounded by yellow fat body; each testis is composed of approximately fourteen testicular follicles. Despax (1951) regards the family Leptoceridae as characteristically having a greater number of testicular follicles per testis, thus they may attain or even exceed twenty per testis. In this species the follicles are associated in a common peritoneal sheath (Fig.60 a p.sh.). Despax maintains that this arrangement is most frequent among Trichoptera. Occasionally however the testes are closely associated by the partial or complete fusion of their outer layer of peritoneum.

Each testicular follicle empties its contents into the vas deferens by way of a very short vas efferens (Fig.60 b v.e.). The vas deferens begins at the anterior end of the testis (Fig.59 v.d.) and is closely applied to it laterally, for some distance. The vas deferens and vesicula seminalis form a slightly convoluted tube leading back to the muscularised ejaculatory duct (Fig.59 vd., vs., ej.d.). The vesicula seminalis occupies a short portion of the lower part of the vas deferens (Fig.59). Transverse sections of the vas deferens just before the vesicula seminalis show that the wall of the duct is more deeply stained

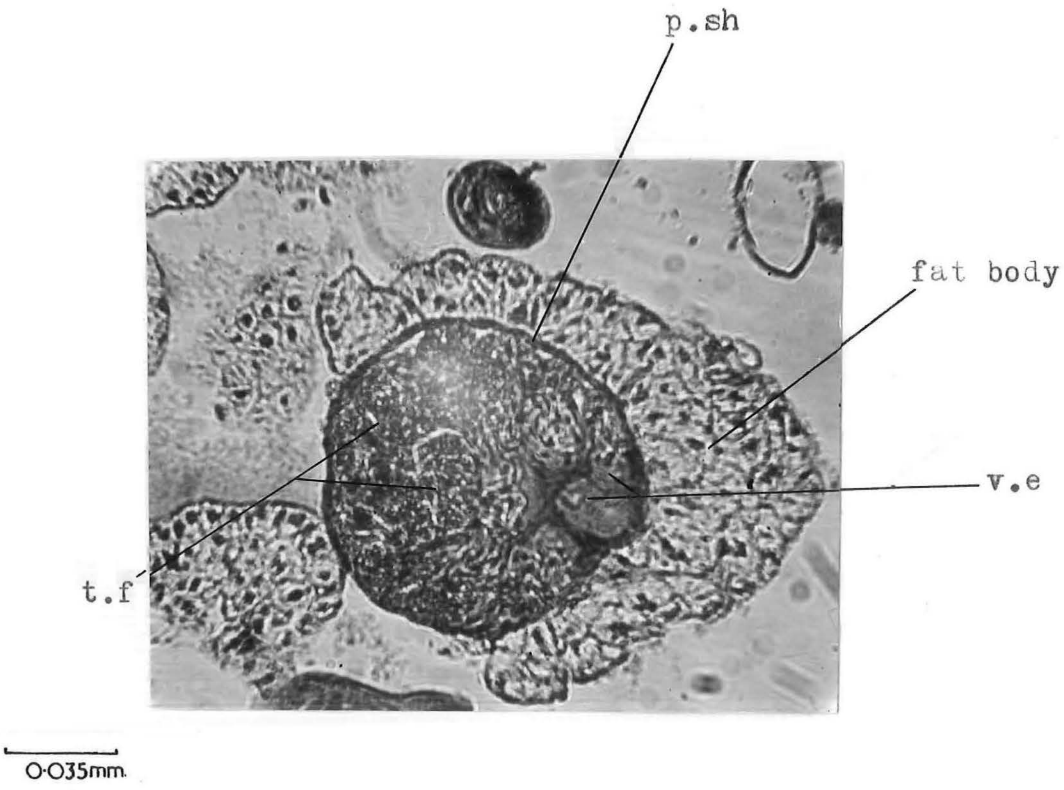
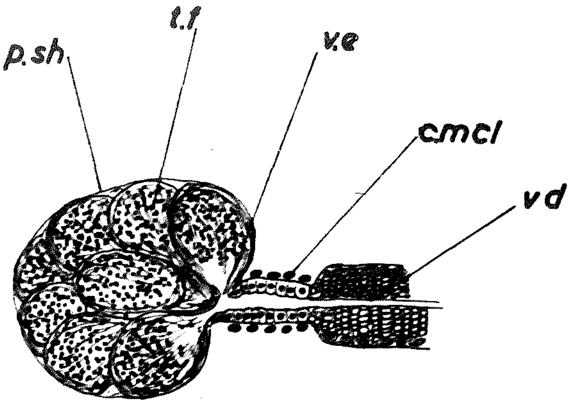
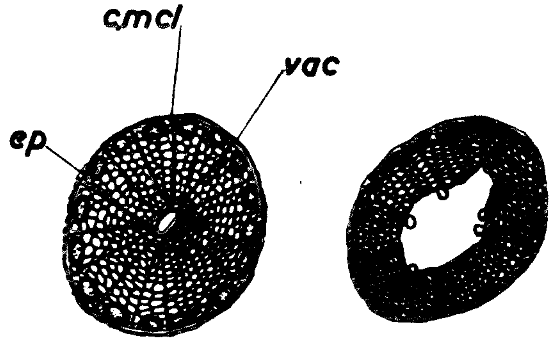


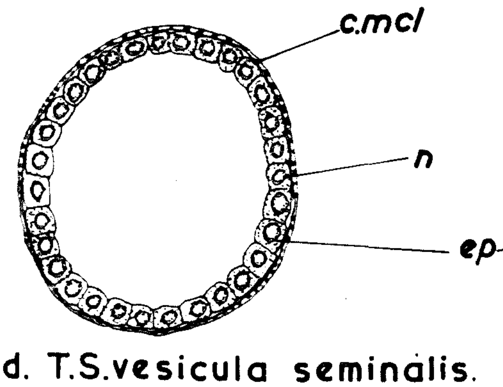

Fig.60a T.S. through testis.



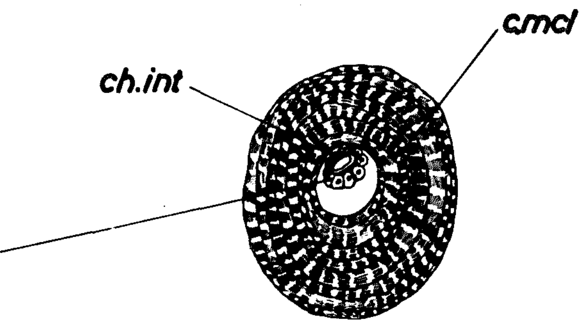

b. L.S. testis, vas efferens and vas defferens. x 600




c. T.S. vas defferens x600




d. T.S. vesicula seminalis. x600

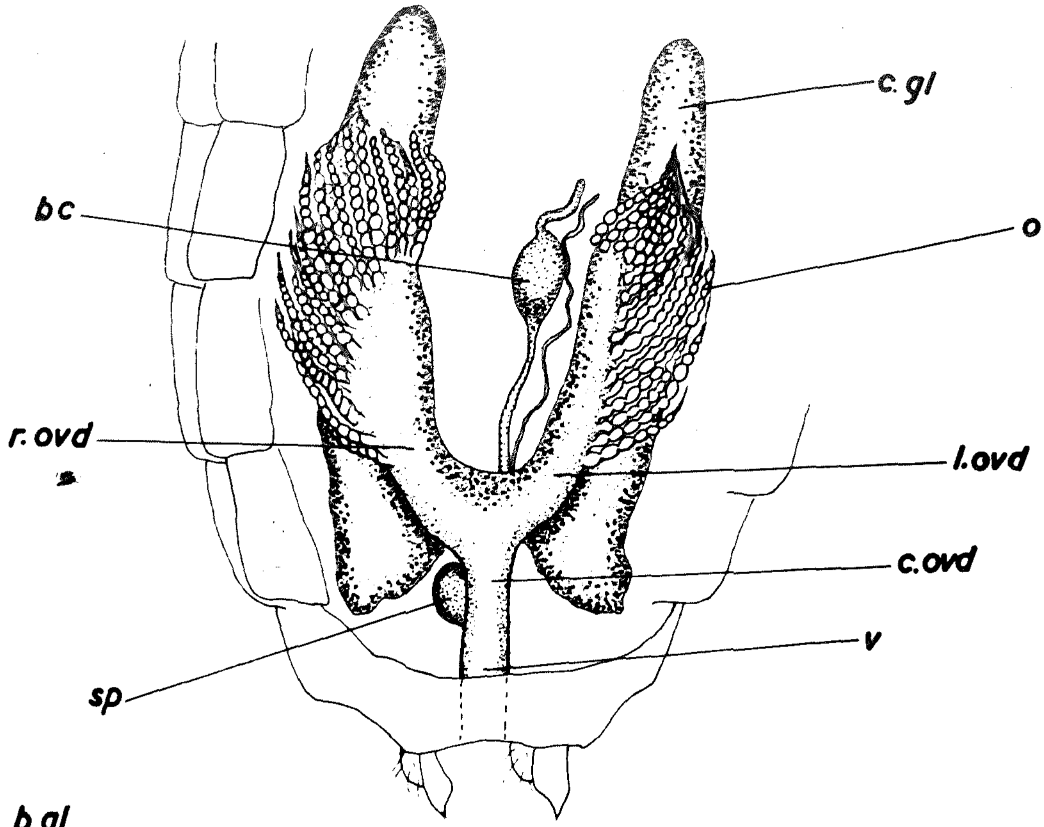



e. T.S. ejaculatory duct. x600

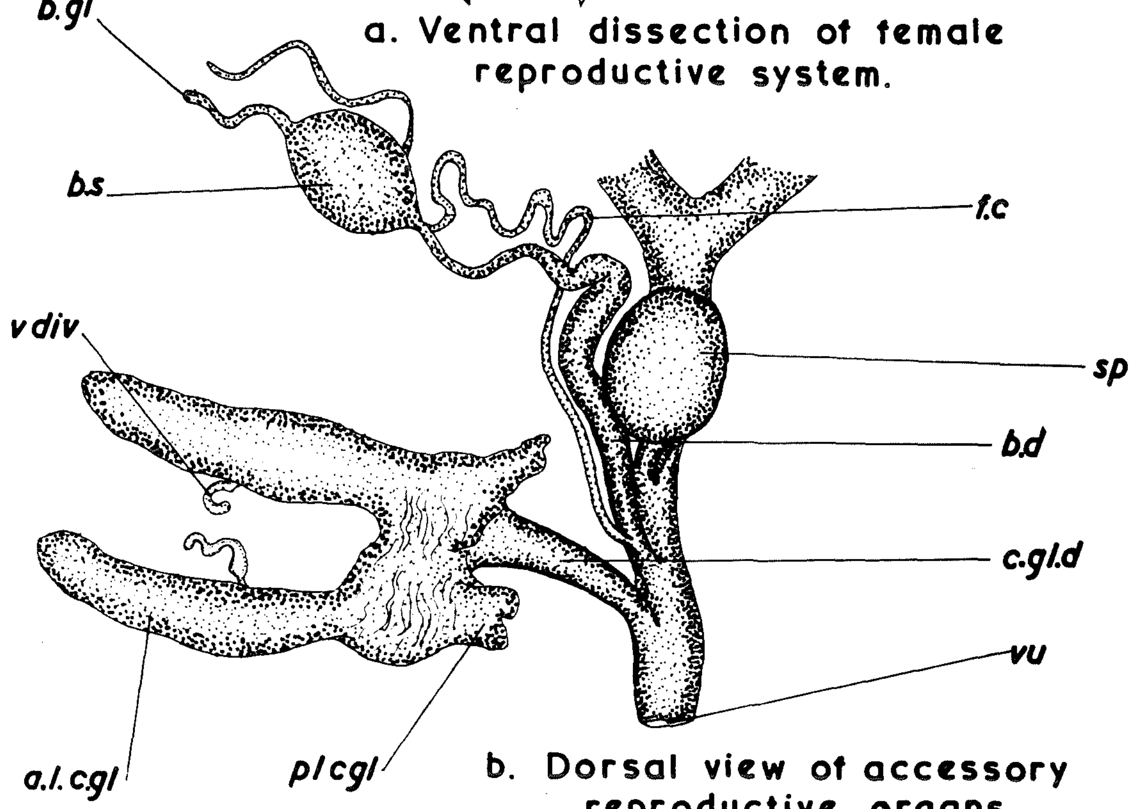
Fig. 60.

than the rest of the vas deferens, and on the free cell border are seen globules which stain in a similar manner to the cell contents of this area. These globules may be some secretion, and this differentiated area may have a similar function to an accessory gland, otherwise absent in this species. The secretions of accessory glands are in some cases said to mix with the spermatazoa (Imms, 1957).

The walls of the vesicula seminalis are quite dissimilar from the vas deferens. In the latter the wall is composed of an outer thin layer of circular muscles. Inside this and arranged round the periphery (Fig. 60 c) are the cells of the epithelium (ep.) with prominent nuclei. From each epithelial cell there is a line of vacuoles which project into the centre so that the lumen is represented by a fine slit. The nature and function of this thickened wall is uncertain, and no contents could be seen in these lines of vacuoles (Fig.60 c vac.) which give a rigid appearance to the tube. The vesicula seminalis (Fig.60 d) has an outer thin layer of circular muscles and a thin layer of epithelium, the cells of which are cubical and have large nuclei (n.). The contents of the vesicula seminalis stained a deep blue with Heidenhains Azan; this is also the colour of the secretory globules seen in the differentiated area of the vas deferens. Behind the vasicula seminalis the vas deferens continues for a short distance, the ducts from each side join to form a common tube which passes at right angles into the ejaculatory duct (Fig. 59 ej.d.). This is composed of a thick layer of circular muscles (Fig. 60 e c.mcl.), a layer of epithelial cells (ep.), with a thin chitinous intima surrounds the lumen. The thick circular muscle layer terminates




a. Ventral dissection of female reproductive system.




b. Dorsal view of accessory reproductive organs.

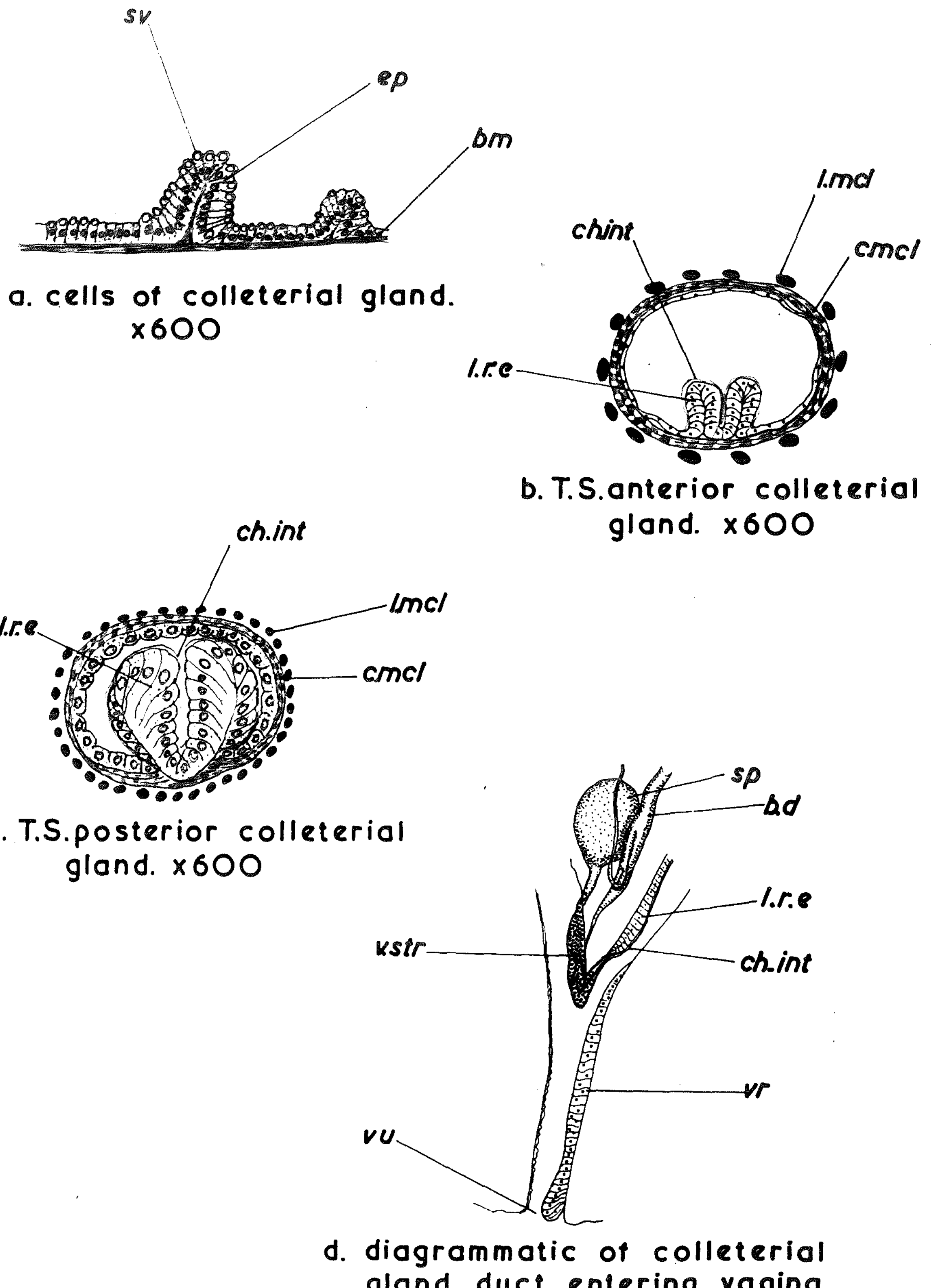
Fig.61

at the proximal end of the phallus, after which the ejaculatory duct is a thin walled tube, with a layer of epithelial cells and a layer of several longitudinal muscle bands.

At the proximal end of the phallus are inserted three pairs of muscles. These are indicated in Figure 59, and the anterior and posterior phallic muscles are responsible for the eversion of the phallus at the time of copulation.

iv. The Female Reproductive System.

The paired ovaries consist each of from 40 - 50 polytrophic ovarioles (Fig.61 a ovl.). In an immature female before any of the eggs have been released into the oviduct, the ovary extends from the anterior end of segment six, to the posterior end of segment seven. The general disposition of the reproductive organs in an immature female is seen in ventral view in Figure 61. The left and right oviducts (l.ovd., r.ovd.) join in segment eight to form a U-shaped structure. From the base of this the common oviduct (c.ovd.) extends posteriorly. After receiving the duct from the spermatheca (s.p.) the common oviduct is then succeeded by the vagina (v.) and this then receives the ducts of the bursa copulatrix (b.c.) and colleterial gland (c.gl.). This relationship is shown in Figure 61 b where the vagina is seen to continue then to the external opening or vulva (vu.) (Snodgrass 1935). This is situated posteriorly on segment nine and guarded on each side by the sclerotised lamellae. Lying dorsal to the ovary are situated the two anterior lobes of the colleterial glands (Snodgrass) (Fig.61 b a.l.c.gl.) each with a ventral diverticulum (v.div.). The extent of the lobes is variable, depending




a. cells of colleterial gland. x600

b. T.S. anterior colleterial gland. x600

c. T.S. posterior colleterial gland. x600

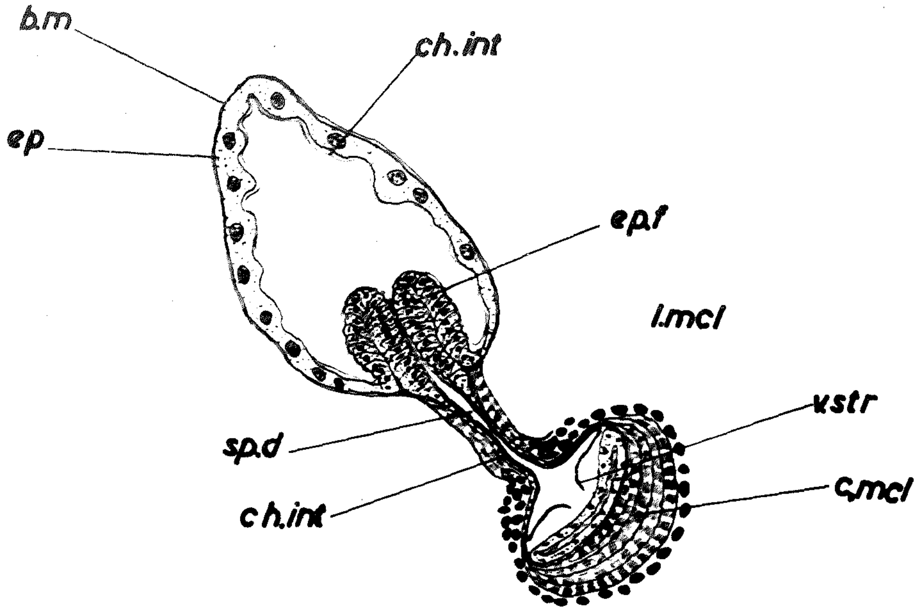
d. diagrammatic of colleterial gland duct entering vagina. (lateral view)

Fig. 62.

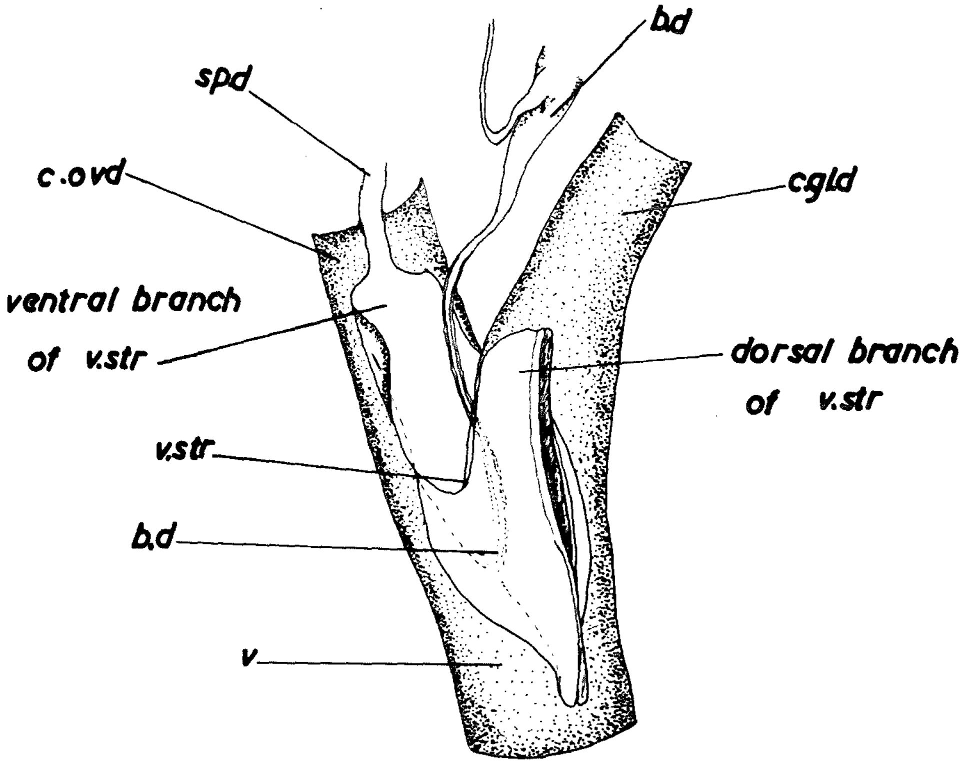
largely on the state of development of the ovary. In Figure 61 a the gland is seen to extend from segment five posteriorly to the postero-lateral lobes (pl.l.c.gl.) in segment nine. In a more mature specimen when most of the eggs have been released into the oviducts, the colleterial gland may extend the full length of the abdomen. The gland is then distended with its secretion which is a gelatinous substance, released at the time of oviposition, and surrounds the eggs to form the egg mass (Dodson 1935, Snodgrass). The gelatinous substance swells considerably when in contact with water. The lobes of the colleterial gland unite to form a duct (Fig.61 b c.gl.d.) which enters the oviduct dorsally in segment eight.

The spermatheca (Fig.61 b sp.) is a globular shaped body, usually in segment eight and enters the vagina by way of a short duct. The bursa copulatrix is a composite structure, consisting of a distal bursal gland (b.gl.), bursal sac (b.s.) a flagellate caecum (f.c.) and the long bursal duct (b.d.) which enters the vagina between the spermathecal duct and that of the colleterial gland.

The walls of the colleterial gland are furrowed and thin. There is an outer basement membrane and an inner layer of folded epithelium. The secretory activity of the epithelium is very evident, as clear vesicles can be seen protruding from the free cell border (Fig.62 a). The colleterial gland duct (Fig.62 b) is much larger than the remaining ducts of the female reproductive system. The wall of the duct consists of an outer longitudinal muscle layer (l.mcl.), an inner circular muscle layer (c.mcl.), and a thin layer of epithelium (ep.). Ventrally the epithelium




a. Spermatheca and spermathecal duct entering vagina.

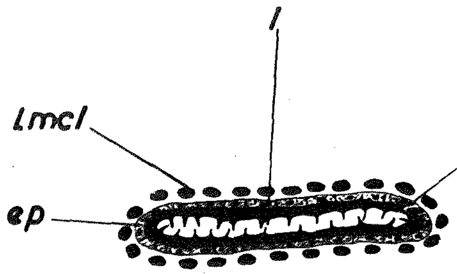



b. Diagram of vaginal structure and associated ducts.

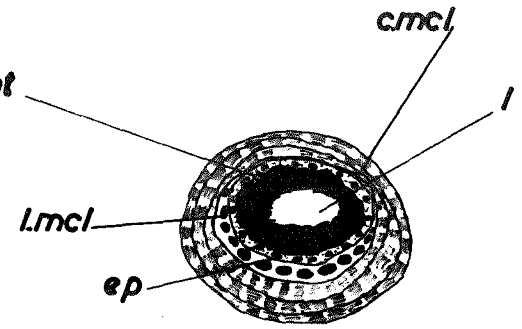
Fig. 63.

is produced to form two longitudinal ridges of thickened epithelium (l.r.e.). Each ridge and the groove formed between them is lined with a chitinous intima (ch.int.), which is continuous with that of the chitinous vaginal structure (Fig.62 d). In the lower portion of the colleterial gland duct these thickened epithelial folds almost obliterate the lumen of the duct (Fig.62 c)., so that when the circular muscles contract, the flow of the gelatinous secretion is efficiently controlled. While the ventral wall of the duct terminates in the dorsal arm of the vaginal structure (Fig.62 d), the dorsal wall in continuous with the dorsal wall of the vagina.

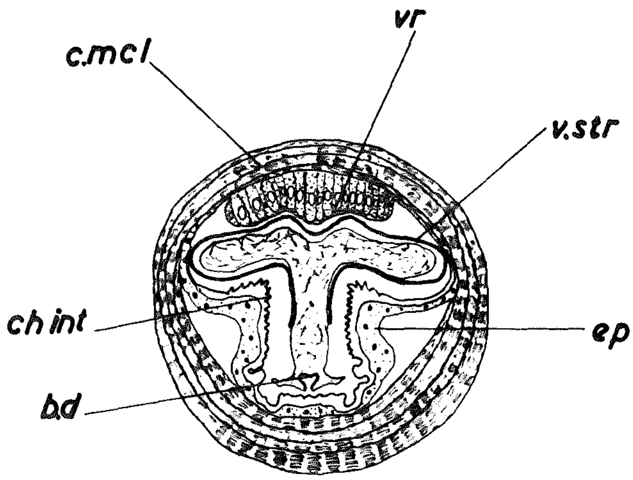
The spermatheca is a saclike organ consisting of an outer basement membrane (Fig.63 a b.m.), an epithelial layer which is very irregular in outline (ep.), and an inner very thin chitinous intima (ch.int.). The opening from the spermatheca is surrounded by a thickened fold of epithelium (ep.f.). The spermathecal duct passes from the opening to the anterior end of the main portion of the vaginal structure into which it opens (Fig.62 d). The duct is lined with a thick chitinous intima (Fig.63 a ch.int.) which is continuous with the tanned chitin of the vaginal structure. Surrounding the chitinous intima there is a layer of epithelial cells and finally a comparatively thick layer of longitudinal muscles (l.mcl.). The base of the spermathecal duct enters the vagina at a parallel angle, the longitudinal muscle layer being continuous with that of the common oviduct and vagina. The entrance of the spermathecal duct according to Snodgrass (1935) marks the termination of the common oviduct and the beginning of the vagina. The spermathecal duct may be



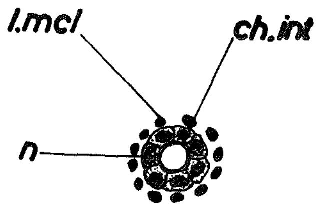

a. T.S. Anterior region of bursal duct. x600




b. T.S. Posterior region of bursal duct. x600




c. T.S. Vaginal structure indicating opening of bursal duct. x150




d. T.S. Flagellate caecum. x600

Fig. 64.

seen from transverse sections to open into the very anterior end of the vaginal structure, which then opens into the vagina (Fig.63 b).

The bursa copulatrix extends forward into the sixth segment, where it ends in the bursal gland (Dodson 1935) (Fig.61 b b.gl.). This has the same appearance in cross section as the flagellate caecum which will be described later. The bursal sac (b.s.) is composed of a thin wall of epithelium which is irregular in outline and is lined with a chitinous intima. The sac is filled with a substance, the exact nature of which has not been determined. Leading from the posterior end of the bursal sac is the thin bursal duct. It is at first flattened with an outer longitudinal layer of muscles, a thin layer of epithelium and finally a chitinous intima, which is covered with numerous fine projections (Fig. 64 a). More posteriorly the duct is round (Fig.64 b) with a very thick layer of chitin. The duct enters the dorsal wall of the vagina (Fig.63 b bd.) where it continues posteriorly within the vaginal structure on a median raised fold of chitin. From here it opens into the vagina (Fig.64 c). Connected to the bursal duct near its base is a long flagellate caecum (Despax 1951), (Fig.61 b f.c.). It is of an even diameter and of the same composition throughout. There is an outer layer of longitudinal muscles (Fig.64 d l.mcl.) and a layer of epithelial cells with comparatively large nuclei (n.). There is a thin chitinous intima.

The common oviduct is composed of an outer layer of longitudinal muscles and a thick band of circular muscles (Fig.65 a l.mcl., c.mcl.). There is an inner epeithelium (ep) lined with a chitinous intima (ch.int.). The lateral oviducts lack the circular muscle layer and the chitinous

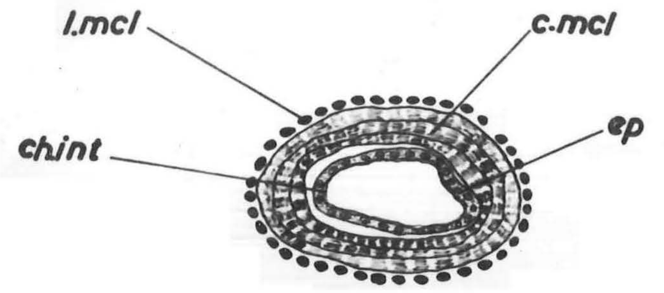



Fig.65.a. T.S. common oviduct.

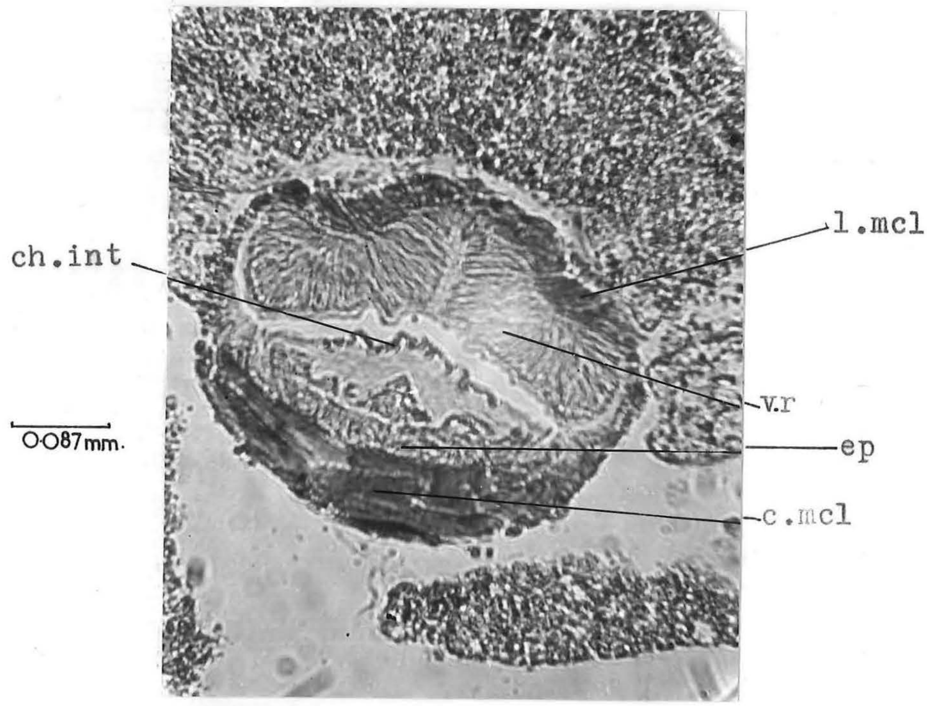



Fig.65b T.S. through vagina to show folding of chitin on ventral ridge of epithelium.

intima. The vagina anteriorly has the chitinous vaginal structure which occludes most of the lumen (Fig.64 c v.str.). It is seen in situ in Figure 66. The wall of the vagina consists of a continuous outer longitudinal muscle layer (Fig.65 b l.mcl.) and a discontinuous layer of circular muscles (c.mcl.). Along the entire length of the dorsal wall of the vagina the epithelium together with a thin chitinous intima forms two longitudinally thickened ridges (Fig.66 a and 65 b v.r.). These originate just above the vaginal structure and diverge around it as they pass poasteriorly, so that the vaginal structure fits into the groove formed between each ridge. Posteriorly right at the vulva, or external opening of the vagina, the ridges converge, (Fig.66a) and form a rounded end. Ventrally the circular muscles form a thicker band than dorsally (Fig.65b,c.mcl.). There is a slight thickening of the epithelium covering which there is a chitinous intima, the outer edge of which is tanned and plicately folded (ch.int.); more posteriorly this feature is lost. The opening of the vagina to the exterior is controlled by a thickllayer of circular muscles ventrally. These are inserted laterally on apodemes of the sclerotised lamellae (Fig.66 b). The function of the longitudinal vaginal ridge and the raised epithelium on the ventral wall of the vagina would be to aid the occlusion of the lumen, when the large circular muscles are contracted.

Dodson (1935) describes Goera pilosa, and Brachycentrus subnubilus, the bursa as being lined with thin chitin very different from the heavy armour in the spermatheca. As can be seen from the above description and illustrations, Dodson's observations do not apply to the same organs in

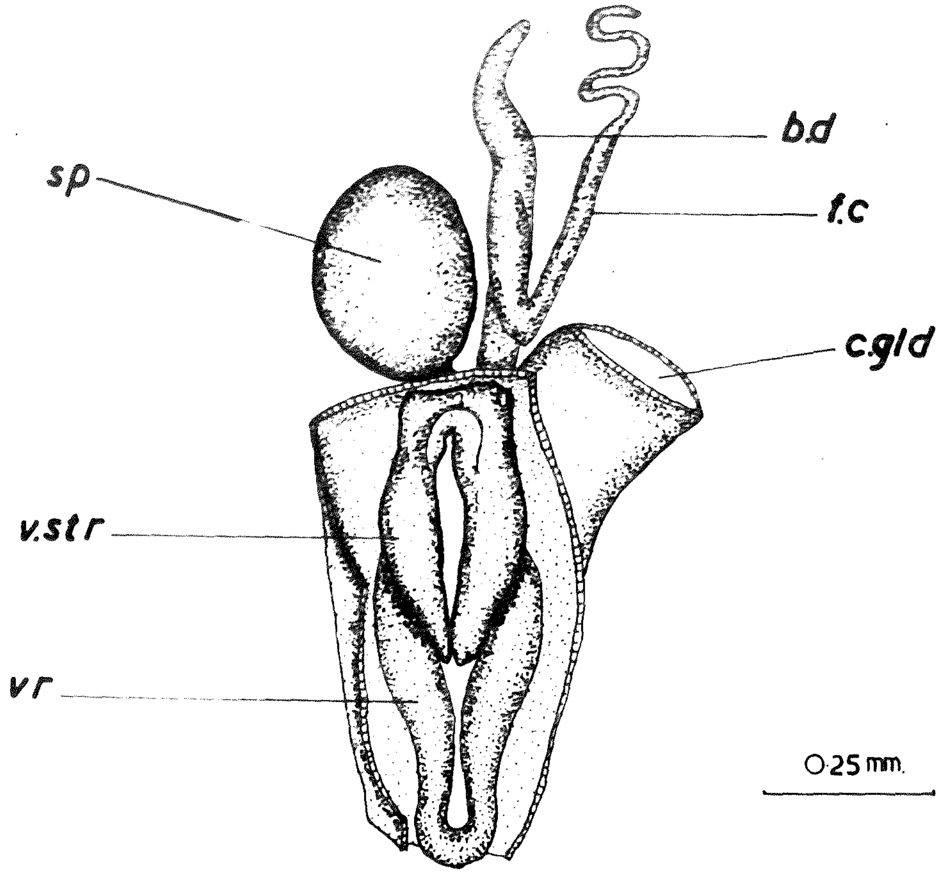



Fig.66.a. Ventral dissection of vagina.

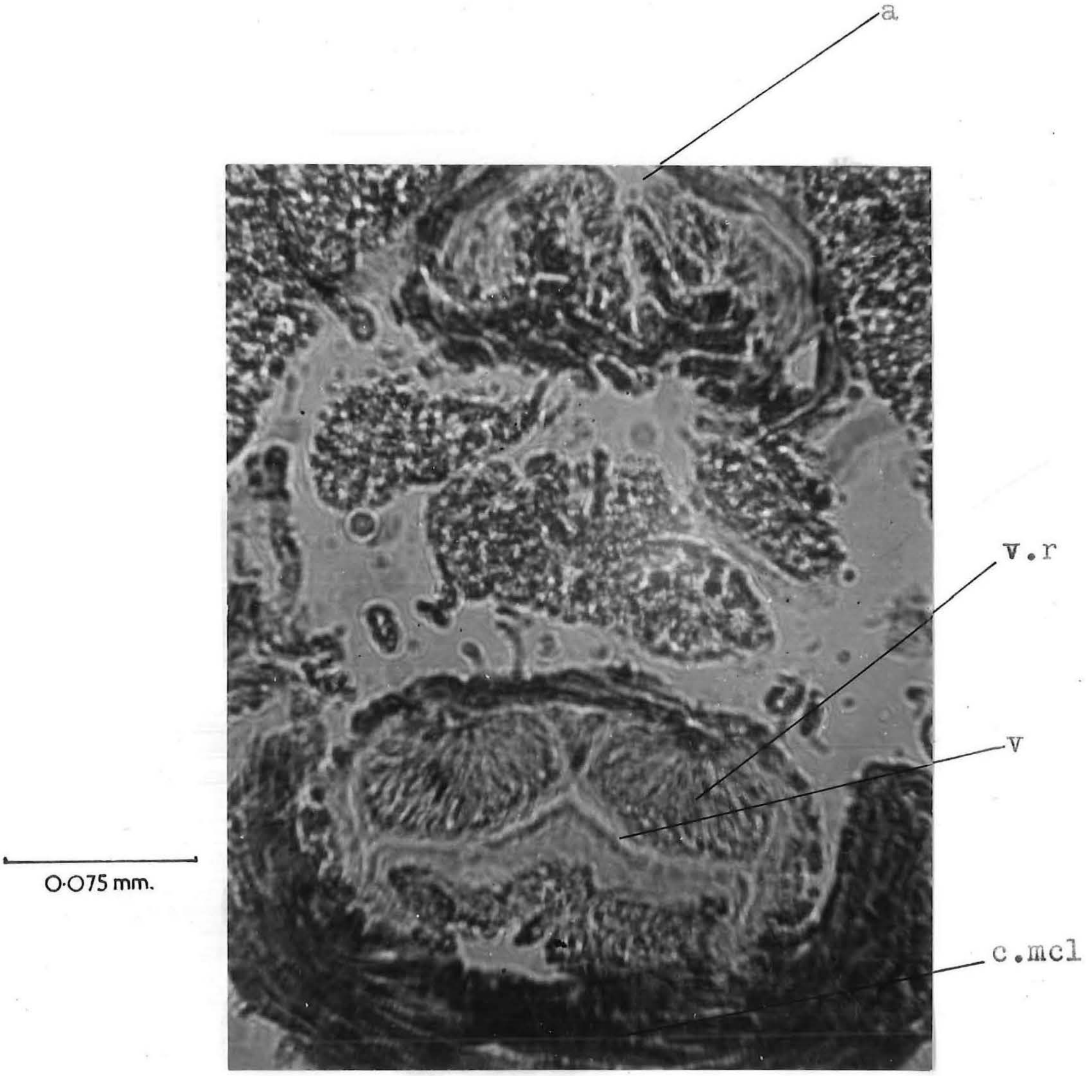



Fig.66b T.S. through segment nine of female to show relative position of vagina and anus.

this species. This fact discounts to a certain extent, the histological support of his view that the bursa copulatrix and spermatheca of Lepidoptera and Trichoptera are not homologous. For he says "the relative position of the Trichopteran bursa and spermatheca is the reverse of that in young stages of Lepidoptera. The names of the Trichopteran bursa and spermatheca should therefore be reversed in order to homologize with the Lepidoptera".

There occurs one main difference in the literature concerning the structure here named bursa copulatrix, and which with one exception is usually indicated as such by other authors. This exception is made by Deoras (1944) who refers to it as the shell gland, but does not give any indication of its exact function or his reasons for calling it shell gland.

It is evident from a review of the literature, that any detail on the anatomy and function of the female reproductive system in Trichoptera is apparently lacking. Apart from Dodson's work on the development of the female genital ducts description is confined to the general form of the reproductive system. If such a description is to be valid and the organs correctly identified it is of primary importance that detailed anatomy and a knowledge of the function of these organs should be obtained.

No mention has been made in the literature of the exact function of the chitinous vaginal structure. From observations made on the female of this species it would seem that this structure has developed in very close association with the openings of the various ducts of the reproductive system. It separates the duct of the colleterial gland from the vagina and provided support for the opening of the spermathecal and bursal ducts.

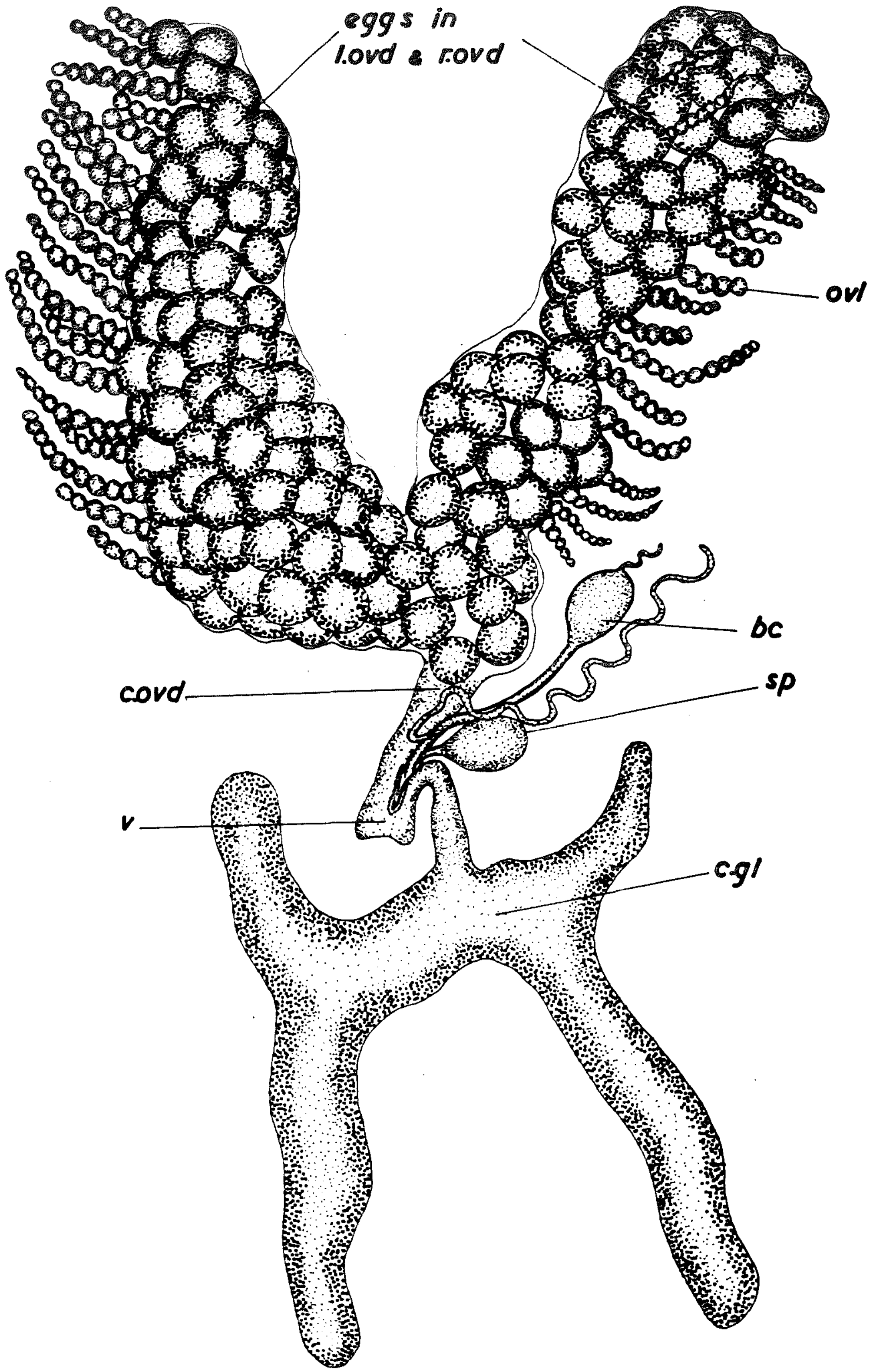
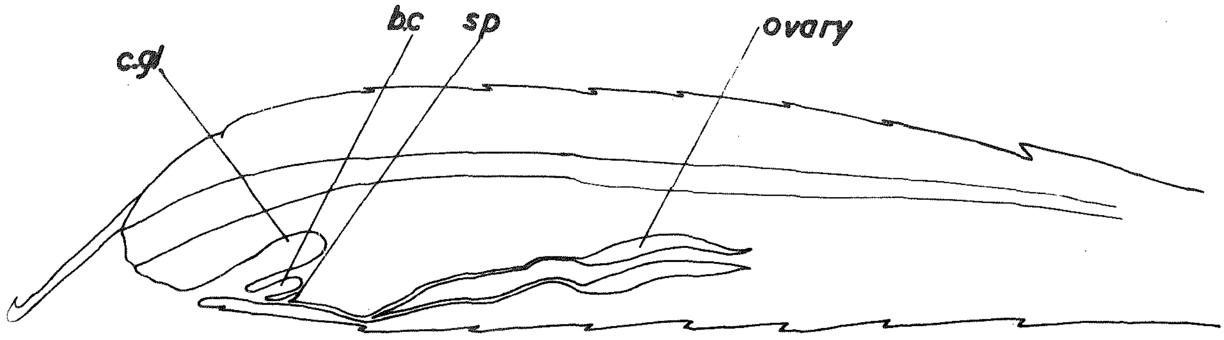



Fig. 67. Reproductive organs of nearly mature female.

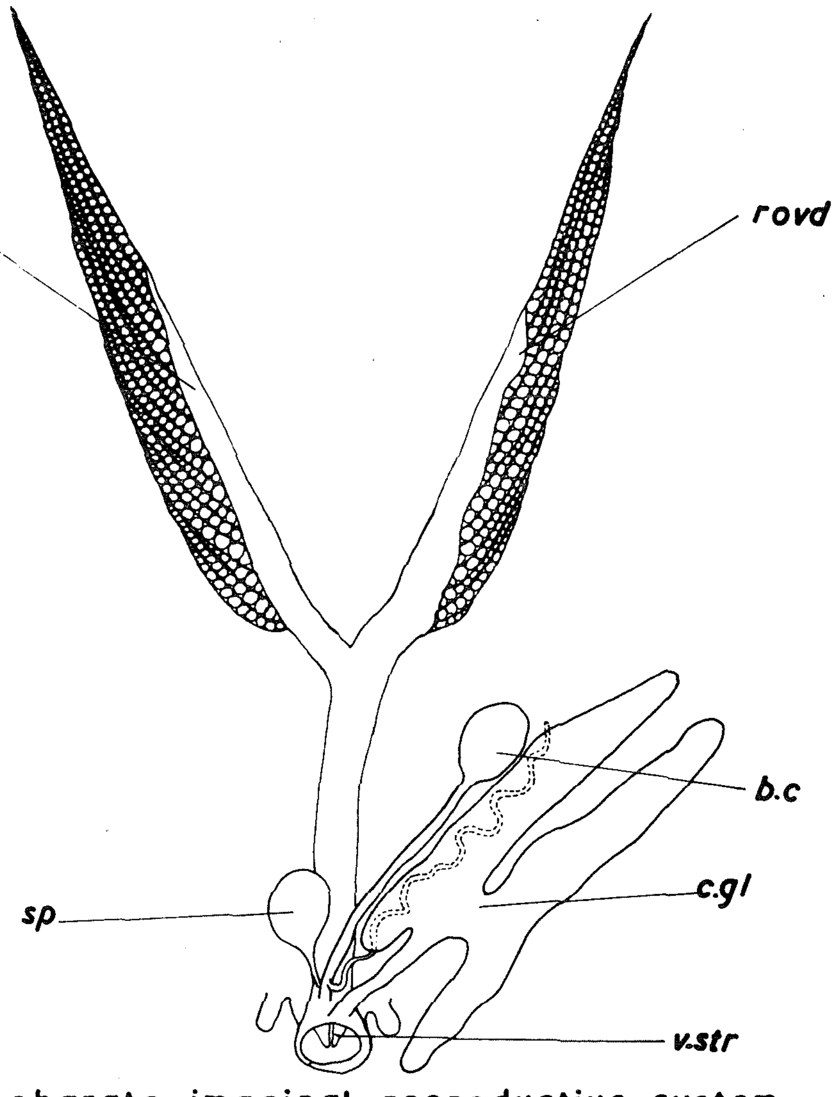
Finally as will be seen in the following paragraph it may be of functional importance at the time of copulation.

The function of the colleterial gland has already been indicated. Of the function of the spermatheca and bursa copulatrix, only tentative suggestions may be made. From an anatomical point of view it would seem likely that the bursa copulatrix in this species retains its normal function, that is, it is a diverticulum of the vagina serving as a copulatory pouch and the sperm being later transferred to the spermatheca. Therefore these structures would be indeed homologous with those of the Lepidoptera contrary to Dodson's conclusions. This view held here is supported mainly because of the fact that the form of the male phallus, especially the nature of the short endophallus would be structurally incapable of reaching the anterior portion of the vaginal structure where the spermathecal duct enters. The opening of the bursal duct however, on a raised median portion of the vaginal structure at its posterior end, would be more likely to receive the sperm from the endophallus. The anatomy and histology of the spermatheca in this species seems to indicate that it is similar to that of the spermatheca as described by Snodgrass (1935) and has the function of storing sperm after copulation.

In the nearly mature female the eggs are ripe and nearly all liberated into the oviducts (Fig.67) which now occupy the greater part of the abdominal cavity. The alimentary canal becomes pushed out of place and appears to diminish in size, although this is probably due to a decrease in activity. The colleterial gland increases in size with the maturing of the ovary. Finally in the fully mature specimen, the oviducts with




a. pharate pupal reproductive system.




b. pharate imaginal reproductive system.

Fig.68.

their eggs extend from the first to the seventh abdominal segment. They are so packed in the abdomen that the left and right oviducts lose their identity and the terminal filaments of the ovarioles can hardly be traced. The colleterial gland extends from the first to the ninth segment, the posterior lobes completely filling segment eight and nine. The average width of the eggs in the oviduct is 0.290mm, with a minimum of 0.260mm and a maximum of 0.330mm, as measured to the nearest 0.01mm with a micrometer eye piece. The development of the egg within the ovarioles is shown in Figure 68 c and is characteristic for a polytrophic type of ovariole.

The development of the female reproductive system was briefly examined. The first signs of the early stages, seen by gross dissection, were found in the pharacte pupa (Fig.68 a). The form of the various structures became more apparent by the time the end of the pupal and early pharate imaginal stages are reached. Figures 67 & 68 b were drawn to the same scale by using a squared eyepiece. These figures indicate clearly that the later stages of growth take place almost exclusively in the ovary and colleterial gland.

In the early pharate imago the vagina is short compared with that of the imago. This is seen in Figure 68 b, where the vaginal structure (v.str.) almost protrudes from the vulva, and the ducts from the various organs enter the vagina posteriorly. Consequently before the situation in the adult is assumed, there must occur a further invagination. That this may indeed be so is shown by the fact that the lamellae, which later become sclerotised, are situated anteriorly to the pharate imaginal vulva

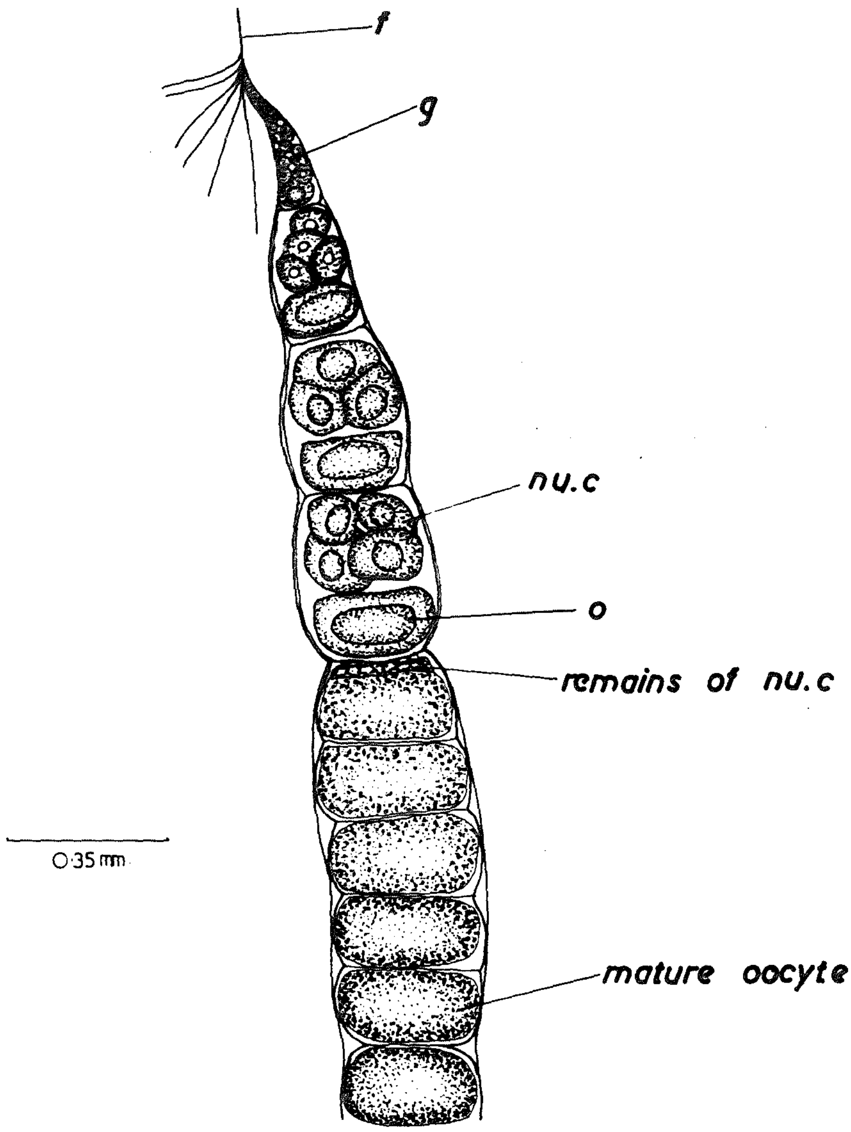



Fig. 68c Development of the egg

(Fig.68 b). Since these sclerotised lamellae of the adult are situated in an extreme posterior position, the difference in position is invaginated, thus forming the adult vagina (Fig.61 b).

## 3/4 ABBREVIATIONS

ac.gl. accessory gland
ant.int. anterior intestine
ant.ph.m. anterior phallic muscle
a.sd. aperture of silk duct

b.ca. buccal cavity
b.c. bursa copulatrix
b.d. bursal duct
b.gl. bursal gland
b.m. basement membranes
b.s. bursal sac

c.gl. colleterial gland
ch.int. chitinous intima
cl.ep. colonic epithelium
c.mcl. circular muscle
conn.t. connective tissue
c.ovd. common oviduct
cr. crop

d.t. directing tube

ep. epithelium
ep.f. epithelial fold
ej.d. ejaculatory duct

f. terminal filament
f.c. flagellate caecum
fg. foregut

g. germarium
gp. gonopore

h. hairlike processes
hg. hind gut

inf.m. inferior muscles

l. lumen
ll. lamella
l.mcl. longitudinal muscle
l.ovd. left oviduct
l.r.e. longitudinal ridge of epithelium

mg. midgut
mid.s.gl. middle region of silk gland

mt. malpighian tubule

n. nucleus
nu. nutritive cells

oes.inv. oesophageal invagination
ovl. ovariole

ph. pharynx
p.int.r. posterior intestinal ring
pl.l.cgl. posterolateral lobe of colleterial gland
post.ph.m. posterior phallic muscle
pr. proventriculus
p.sh. peritoneal sheath
p.t.m. peritrophic membrane
py.s. pyloric sphincter

r. rectum
r.ovd. right oviduct
r.p. rectal pads

s.c. silk core
scl.l. sclerotised lamella
s.d.gr. groove in silk duct
s.g. salivary gland
s.g.d. silk gland duct
si.gl. silk gland
sp. spermatheca
s.p. silk press
sp.d. spermathecal duct
sph. sphincter
str. b. striated border
sup.m. superior muscles
s.v. secretory vessicles

t. testis
t.f. testicular follicle

v. vagina
vac. vacuole
v.d. vas deferens
v.e. vas efferens
v.r. vaginal ridge
v.s. vesicula seminalis
v.str. vaginal structure
vu. vulva

# Chapter 4: SOME ECOLOGICAL OBSERVATIONS.

## 4/1 The Habitat.

### i. Introduction.

Ecological considerations in this chapter are almost entirely confined to those associated with the aquatic stages of Triplectides obsoleta, especially the larval stages. An indication of the broader aspects of the habitat has previously been noted in the Introduction and subsections on the Life History. From observations made on the three main habitats in which the larval stages have been taken, Upper Styx, Lower Styx, and the tributary of the Hawdon River near Cass, one obvious feature is apparent. This is the variety of environmental conditions which the larval and pupal stages are able to withstand.

In the following subsections the substratum and aquatic vegetation will be described for the Upper Styx area; also the surrounding vegetation will be indicated. The physical characters of the stream including the velocity of the water, and temperature relations will be dealt with, and finally some chemical features of the water are described. In conclusion some differences in the habitat of the Lower Styx and the Hawdon areas as compared with the Upper Styx will be discussed.

### ii. The Substratum.

In the type locality, near the source of the Upper Styx, the bottom is mainly silty. Where the silt cover has been abraded away the gravels are exposed and give a firm substrate. The plant debris is usually only deposited in any quantity over the silted parts of the stream as these are deeper and quieter waters than those which flow over the gravel stretches. From observations made during the year, it seemed that the amount of debris in the stream reached a maximum during the autumn and early winter, after which the amount decreased until replenished the following autumn.

iii. Aquatic Vegetation.

The macroscopic vegetation is externely sparse. *Elodea canadensis* and *Nitella* sp. are the most common species present. These plants form matts of vegetation on the beds of silt and provide a browsing ground for several aquatic species including *Triplectides obsoleta*. The microscopic vegetation was not examined in detail.

iv. The Surrounding Vegetation.

Species of higher plants that give cover to the stream have previously been listed. Beneath this cover there is another layer of vegetation consisting of various species of tall grasses, ferns and a dense cover of *Convolvulus* sp. This lower layer of vegetation overhangs the water and would, in time diminish the width of the stream if it were not for the fact that twice a year the vegetation is cut back to form a straight sided bank.

v. Velocity of the Water.

The velocity of the water in the stream was measured by using a cork float and timing it to the nearest 0.5 of a second over a known distance by means of a stop watch. Using this method it was only the velocity of the water in the centre of the stream which could be calculated. A number of recordings were made and the average of these gave the velocity as being 1.16 feet per second. It is interesting to note that Hynes (1960) records a table reproduced from Butcher (1933) which tabulates the relation

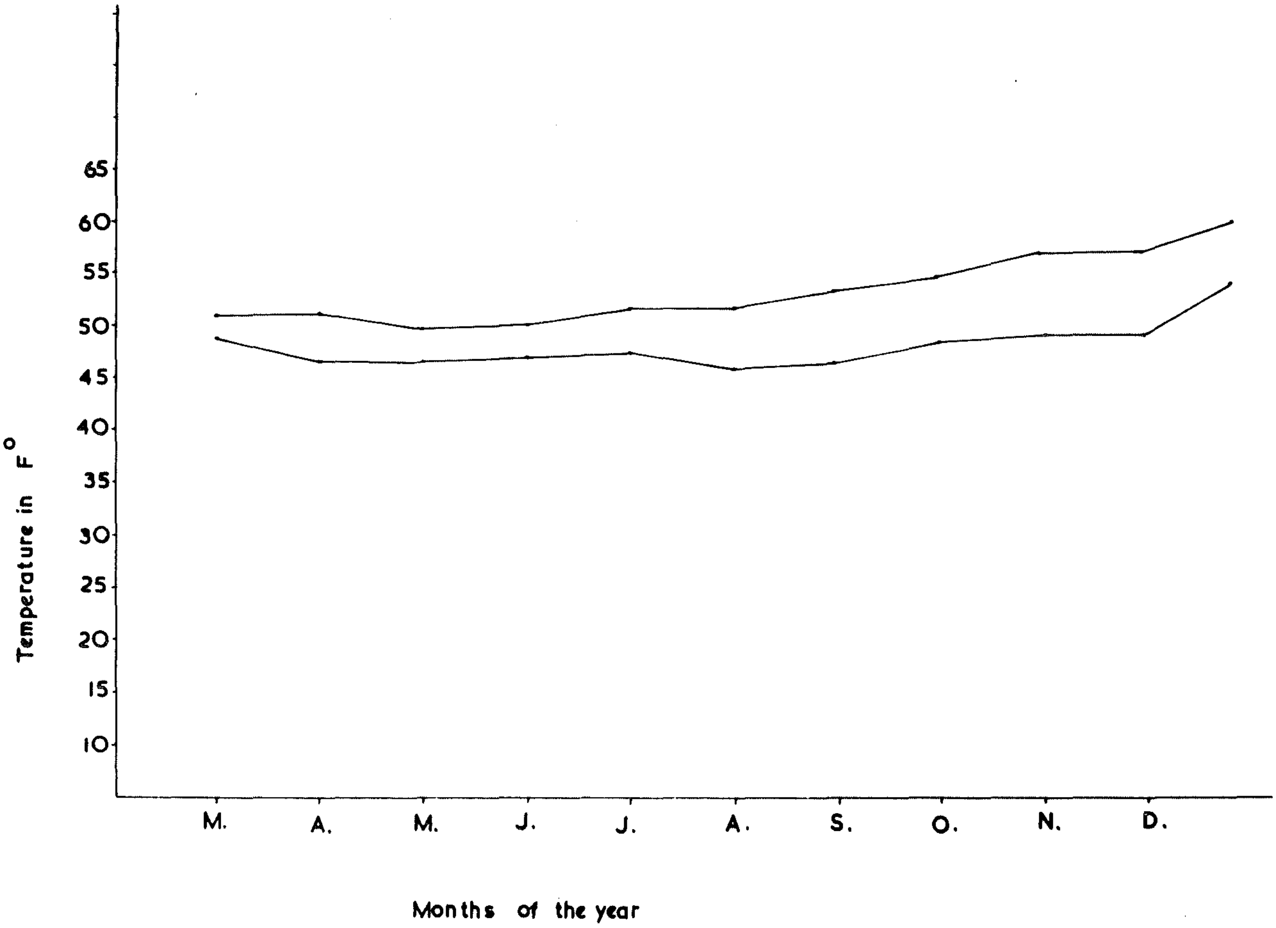



Fig.69. Maximum minimum temperature recordings of water for upper Styx.1961.

of current speed and the nature of the river bed. In this the nature of the bed corresponding to the velocity of more than one foot per second is gravel and the type of habitat is partly silted. This is of course only a very general estimation as the current is not uniform, nor is it always constant.

The velocity of the stream was calculated merely to give a more complete picture of the habitat, and also for comparative purposes. The effect of the current on the aquatic stages was not dealt with. It is of interest however, that emergence appears to have taken place only in the running water aquaria, it has not been observed in the stillwater tank.

vi. Temperature Relations.

The temperature of the water in the Upper Styx varies within only a few degrees from summer to winter, and the maximum and minimum have a narrow range. Unfortunately continuous recordings were interferred with, by the fact that maximum and minimum thermometers were twice removed from the Upper Styx. Continuous recordings were taken from March until December. However, isolated readings taken during January and February indicated temperatures of from 10°C to 13°C which is in accord with the moderately stable temperature conditions as shown in Figure 69.

In the laboratory some experimental aquaria were set up at different temperatures. These were all several degrees above that of the natural habitat and averaged between 18°C and 20°C. A number of larvae were placed in each aquarium and air was bubbled through the water continuously. Adequate plant material was supplied. It was hoped that by increasing the temperature, the time taken for each instar might be decreased so that finally the onset on pupation would occur earlier than under normal temperature conditions. It is difficult to measure head widths of larvae when alive, consequently the exact termination of each instar could not be accurately determined. However one aquarium containing 2nd and 3rd instars at the beginning of June, contained by the beginning of August 3rd and 4th instars. This is the time which has already been calculated to be the approximate duration for the 3rd, 4th and 5th larval instars (Chapter 1). It seems therefore that the increased temperature does not have any obvious effect on the duration of these instars.

Together with the above experiments some observations were made on larvae kept for short periods of up to two months at temperatures of 25°C and 30°C. The aquaria were kept in constant temperature tanks and the water in the aquaria was kept well aerated. The larvae were supplied with an assortment of alive and dead plant material. The larvae were found to tolerate these higher temperatures for the times stated above, providing the water was well aerated. The exact maximum of temperature tolerance was not found.

These observations indicate that the larval stages of Triplectides obsoleta has a wide temperature tolerance and that increased temperature apparently does not have any obvious effect on the duration of these instars. However, more detailed experimental work carried out for longer periods of time, would have to be undertaken before any conclusions could be reached concerning relations between temperature and larval stages.

vii. Some Chemical Features of the Water.

The geographical situation of the Styx River is such that it drains a considerable area of farmland and because of this, it was thought that the amount of organic residues entering the stream as effluents may be significant. Concerning these organic residues and their effects on pollution Hynes says "Fundamentally the basic property of this type of effluent is that it contains unstable compounds which are readily oxidised and so use up the dissolved oxygen in the water". Therefore an excess of organic effluent in this way will ultimately bring about pollution of the area. It was decided that the organic chemical conditions of the Hawdon area and also that of the still water aquarium in the laboratory would give useful comparisons. For the following analyses, sampled on the 3/11/61 and 7/11/61, I am indebted to the Government Analyst, N.P. Alcorn M.Sc., A.R.I.C., of the Dominion Laboratory.

Analyses:-

| | Upper Styx River | Laboratory Tank | Tributary of the Hawdon R. |
|---|---|---|---|
| pH. | 7.0 | 7.5 | 7.3 |
| parts per million | | | |
| Chlorine in chlorides | 7 | 17 | 2 |
| Nitrate nitrogen | 0.4 | 0.1 | nil |
| Nitrite nitrogen | Trace | 0.01 | nil |
| Ammoniacal nitrogen | nil | 0.72 | nil |
| Albuminoid nitrogen | 0.014 | 0.666 | nil |
| Oxygen absorbed in four hours at 80°F. | 0.35 | 0.35 | 0.02 |

According to Hynes the appearance of nitrates in a sample, speaking with special reference to sewage-works effluent, indicates that satisfactory oxidation processes are being carried out. The amount of nitrate nitrogen in these samples would not be enough to cause pollution by decreasing the $O_2$ content. Compared with the Upper Styx, the Hawdon area sample is relatively free from nitrogenous organic matter. The increase in most of the figures from the laboratory tank is due no doubt to the fact that the water is stationary.

It should be remembered that there would be a certain amount of fluctuation in the above figures throughout the season. The analysis seems however to indicate that the condition in the Upper Styx as far as organic matter is concerned is a normal situation. The seepage from any farming activities apparently has no drastic effect.

viii. <u>Some differences in the Lower Styx River and Hawdon Areas as compared with the Upper Styx River.</u>

The stream entering the Hawdon River is very different from the Upper Styx. It is wider, approximately ten to twelve feet across, and of a more even depth throughout, from one to two feet deep. The substratum is stony, consisting of medium sized pebbles and small boulders about one foot across. Only in the small eddies at the edge of the stream is any sediment deposited, and the current sweeps down stream the plant debris of leaves, twigs and branches. In the Upper Styx, on the other hand, all this type of plant debris is deposited on the bottom. Because of the swifter nature of the current and lack of silty substrate there is little in the way of macroscopic aquatic vegetation in the tributary of the Hawdon River. In the Lower Styx area the habitat of the larvae is confined to the sides of the river in the thick growth of <u>Elodea canadensis</u>. The water is deep, slow flowing and seldom clear.

The chemical features of the water for the tributary of the Hawdon River, and as seen in subsection vii, show that the water is relatively free of any organic content; also only 0.02 Oxygen is absorbed in four hours compared with 0.35 for the Upper Styx.

Three isolated temperature recordings from the tributary of the Hawdon were for June, 7.5°C, September 5.5°C, and November 6.3°C. These are much lower than the Upper Styx readings.

## 4/2 Notes on the Associated Fauna of the Upper Styx River.

The following is a very superficial indication of some of the associated forms occurring in the Upper Styx Stream. Only a small number of animals have been identified to the specific level.

The vertebrates commonly occurring consist of the eel, Anguilla sp., whether short or long finned was not determined. The brown trout Salmo trutta, was commonly seen.

Usually found amongst the Elodea was the freshwater prawn Paratya curvirostris. From the Elodea and also from the bottom debris, a considerable number of the small snail Potamopyrgus badia Gould (Suter 1913) occurred. Another mollusc found in the debris was the bivalve Pisidium novae zelandiae.

Amongst the debris of the stream bed and under stones and wood are found a small grey flatworm, and a red oligocheate annelid. Aquatic stages of Ephemeroptera include a species of Deleatidium, Coloburiscus humeralis and a larger species than Deleatidium sp., olive brown in colour, which was not identified. Chironomid larval stages were common and also larval and pupal Culicids. The Trichopteran species present include Olinga feredayi, Oecenis? maori, Oecetis sp., Hydrobiosis parumbripennis, and Polyplectropus purilus.

## 4/3 The Local Distribution of *Triplectides obsoleta*.

Hudson (1904) records the species as having occurred at Auckland and Wellington in the North Island, and at Nelson, Christchurch, Ophir and Invercargill in the South Island.

During the present work, the species has been recorded from the small west tributary of the Hawdon River, near Cass, the South Branch of the Waimakariri River, the Upper reaches of the Heathcote River and the Styx River in Canterbury, and from the Pelorus River in Marlborough.

## 4/4 Discussion and Conclusions.

From the preceding sections we see that *Triplectides obsoleta* has a wide range of habitat, the aquatic stages living in different types of streams, from the deep, slow flowing Lower Styx, to the shallow more swiftly flowing tributary of the Hawdon River. The amount of aquatic vegetation varies considerably in the different areas as does the nature of the substratum. The most significant feature, however, is the temperature tolerance shown by the larval and pupal stages. This was observed in the field and also found by experiments with the larvae, undertaken in the laboratory. The habitat of the larval and pupal stages is however confined to running water.

The features discussed in this chapter do not give any indication as to what could be some of the limiting factors, in the distribution of this species. What is it then that governs its distribution? In Chapter 1 and 2, the wood boring habit with reference to case building and pupation sites peculiar to the larval and pupal stages respectively, was described. From this it follows that the distribution of the species will be largely governed by the presence or absence of woody substrates, in order to meet these special requirements of the various stages in the life history.

## SUMMARY AND CONCLUSIONS.

The major part of this work is concerned with the life history of Triplectides obsoleta, together with some biological and morphological aspects of the larval, pupal and adult stages.

It has been established from field work, that the larval stages are present in the population throughout the year. The growth of the larva continues throughout the winter until the final instar is reached, mainly during the spring and summer months. The larval instars were identified by measuring head widths, and from these larvae were found to fall clearly into five instar classes, from the 2nd to the 6th. By using head width measurements an indication of the number and increase in age of the instars present in a population sample for the months April, until and including October, was acheived. For the succeeding months of the year it was not possible to sample by using the described techniques, as the greater percentage of the larval population, consisting mainly of the last two instars had undergone a shift in microhabitat. The habitat of the egg mass and the 1st instar are yet to be found.

An unusual feature of this species is the characteristic boring habit of the larva especially of the later instars. This phenomenon has led to the utilisation of solid pieces of wood for pupation sites. Prior to this work, information concerning the habitat of the pupal stage was uncertain.

The imago is found from November until the end of March. The flight activity was found to be at a maximum at dusk, during which time emergence also occurred. The length of life of the individual in captivity was found to have a maximum of 19 days, this could well be exceeded in the field.

Liquid was frequently observed to be taken up by the adults kept in captivity. Some of the morphological features especially of the male and female wings and the male genitalia have been described in more detail than others. An attempt to elucidate the homologies and terminology of the male genitalia has been made. Of considerable importance as far as systematics is concerned is the variety of form shown in the male genitalia, also the differences in the male and female wing venation.

An interesting feature of the larval case building habit is the differential selection of material with age of the larva. Sand grains were found frequently incorporated in the cases of 2nd instars, the remaining instars all used assorted material of plant origin, but the 5th and 6th instars showed a decided preference to tunnel out a case from solid woody pieces.

The food of the larva was found to consist mainly of shavings of dead plant material. It is concluded that diatoms and other microflora within or on the dead plant cells constitute the important sources of nutrition for the larva. A correlation of the feeding habits of Trichoptera with the morphological features was discussed. In T. obsoleta it was decided that the correlation of morphological features with the feeding habits lies in the form of the mandibles and the development of bristles on the mouth parts.

The occurrence on the larval stages of symbionts in the form of a rotifer Philodina sp. and a gregarine from the gut were recorded. These are regarded as facultative commensals. Some Protozoa found on the larval exoskeleton were also noted.

The silk glands of the larva are long simple tubes and have, associated with the duct of each gland, a pair of bilobed accessory glands. The detail of the spinning apparatus was investigated together with some functional aspects of this mechanism. The glands and accessory structures were found to differ in certain respects from those of Hydropsyche colonica, as seen by Glasgow (1936). The silk thread emitted from the aperture at the tip of the ligula is double in form, and is used to line the larval case and pupal cocoon. It is also used to bind together the individual pieces in the construction of the case made from assorted material.

From the comparative anatomy of the larval, pupal and adult alimentary canal some interesting differences were recorded in regard to the general form of the gut and also the number and development of sphincters and valves. The detailed anatomy and histology of the adult alimentary canal has not previously been recorded. Descriptions have been published only of the general form of the alimentary canal, in a number of caddisflies. With special reference to function, it is concluded from evidence gained in the investigation of T. obsoleta that the greater development of valves and sphincters in the adult alimentary canal when compared with that of the larva, is closely associated with the different types of food taken in the two stages. The proportions of the different regions of the alimentary canal are shown to differ appreciably from the larval, to the pupal and finally the adult condition. The number and position of the malpighian tubules is constant throughout the life cycle.

The reproductive systems proved an interesting and informative investigation, especially with regard to the female with the development of accessory glands. The anatomy of the male reproductive system seemed to conform with that of species that have been investigated by other workers. In the female, on the other hand, the anatomical detail of the ducts and their entry into the vagina is additional information to that already known. Here reference is made to the epithelial folds of the colleterial gland duct, the invagination of epithelium at the commencement of the spermathecal duct and also the epithelial thickenings along the dorsal and ventral wall of the vagina. Finally the detail of the chitinous vaginal structure is described. It is suggested that this has been developed in order to support the openings of the various ducts and also that it may be of functional importance at the time of copulation.

In the final chapter some ecological aspects have been related. Although these observations are somewhat brief, they indicate some important features. Significant among these is the wide range of habitat of the species and the marked degree of temperature tolerance exhibited by the larval stages. It is these facts together with the known distribution of the species, which leads to the conclusion that it is neither the physical nor the chemical environmental conditions that primarily govern the distribution of this species. Instead it is the special requirements of the larval and pupal stages, for woody substrates, which are necessary for the wood boring habits associated with case building and pupation, that would be of primary importance in governing the distribution of Triplectides obsoleta.

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## REFERENCES.


Allee, W.C., Emerson, A.E., Park, O., Park, T., Schmidt, K. 1949. Principles of Animal Ecology.

Betten, C. 1934. The Caddis Flies or Trichoptera of New York State. with Chapters by B.L. Kjellgren, A.W. Orcutt and M.B. Davis. Bull.N.Y.St.Mus., No.292.

Brindle, A. 1958. Night Activity of Trichoptera. Ent.Mon.Mag., 94: 38-42.

Crichton, M.I. 1957. The structure and function of the mouthparts of adult caddis flies (Trichoptera).

Corbet, P., and Audfinn and Tjonneland, 1955. The flight activity of twelve species of East Africa Caddis Flies (Trichoptera). Univ. Bergen Arbok. Nautr. rekke, 6: 49.

Corbet, S.A. 1950. The larval development and emergence of Aeshna cyanea (Müll) (Odonata: Aeshnidae). Ent.Mon.Mag., 95: 241-245.

Das, G.M. 1937. The musculature of insect larvae. Q.J.M.S., 80: 39-80.

Davis, M.B. 1934. Habits of the Trichoptera. In The Caddis Flies or Trichoptera of N.Y. State by Betten, 1934; see above.

Deoras, P.H. 1944. Morphology and evolution of adult Trichoptera. Indian J. Ent., 6: 35 - 48.

Despax, R. 1951. Ordre des Trichoptères. In Traité de Zoologie. Grassé, P. Tome X, 1er fasc.

Dodds, G.S. and Hisaw, F.L. 1925. Ecology and Respiration of Trichoptera. Ecology 5, pp.137 and 262.

Dodson, M.E. 1935. Development of the female genital ducts in Trichoptera. Q.J.M.S., 77.

Döhler, W 1914. Beiträge zur Systematik and Biologie der Trichoptenen. S.B. naturf. Ges. Leipzig, 41.

Edmundson, W.T. 1959. Ward and Whipple's Freshwater Biology.

Gilson, G. 1893. Cellules sécretantes. La Soie et les Appareils sericigenes: II. Trichoptera.

Glasgow, J.P. 1936. Internal anatomy of a caddis (Hydropsyche colonica). Q.J.M.S. 79.

Grieve, E.G. 1937. In culture methods for invertebrate animals, by Galtsoff, P., Lutz, F., Welch, P., Needham, J.

Hanna, H.M. 1957. A study of the growth and feeding habits of the larvae of four species of caddis flies. Proc. Roy. Ent. Soc. Lond. 32 : 139 - 146.

\----- 1959. The growth of larvae and their cases and the life cycles of five species of caddis flies (Trichoptera). Proc. Roy. Ent. Soc. Lond., 34: 121 - 129.

\----- 1960. Methods of case building and repair by larvae of caddis flies. Proc. Roy. Ent. Soc., 35, parts 7 - 9.

\----- 1961. Selection of materials for case building by larvae of caddis flies (Trichoptera). Proc. Roy. Ent. Soc. Lond., 36: 37 - 47.

Hickin, N.E. 1946. Larvae of the British Trichoptera. Trans. Roy. Ent. Soc. Lond., 97: 187 - 212.

\----- 1949. Pupae of British Trichoptera. Ibid. 100: 275 - 289.

Hinton, H.E. 1946. A new classification of insect pupae. Proc.Zoo.Soc.,116.

\----- 1958. Comcealed phases in insect metamorphosis. Sci.Prog. No.182, pp.260 - 275.

Hudson, G.V. 1904. New Zealand Neuroptera. London

Hynes, H.B.N. 1960. The Biology of Polluted Waters.

Imms, A.D. 1944. On the constitution of the maxillae and labium in Mecoptera and Diptera. Q.J.M.S., 85: 73 - 96.

\----- 1957. A General Textbook of Entomology.

Lestage, J.A. 1921. Trichopteres in Rousseau, E. Les Larves et Nymphes aquatiques des Insects d'Europe. Bruxelles 1921.

Lloyd, J.T. 1915. Wood Boring Trichoptera. Psyche, 22: 17 - 21.

\----- 1921. The Biology of the North American Caddis Fly Larvae. Bull. Lloyd Library 21, Ent. Series 1, 1921.

MacDonald, W.W. 1950. The larvae of <u>Mystacides azura</u> L., <u>Cyrnus flavidus</u> McLachlan and <u>Oxythira simplex</u> Ris. Proc. Roy. Ent. Soc. Lond. 25: 19.

McFarlane, A.G. 1938. Life Histories and Biology of New Zealand Rhyacophilidae Order Trichoptera. Unpublished Thesis, University of Canterbury.

----- 1939. Additions to the New Zealand Rhyacophilidae. Trans. Roy. Soc. N.Z., 69: 330 - 340.

----- 1951. Additions to the New Zealand Rhyacophilidae. Rec. Cant. Mus., Vol. 5, no.5.

McLachlan 1862. Pseudonema obsoleta. Trans. Ent. Soc. Lond., 1: 305 - 6.

Mosely, M.E. 1953. Trichoptera of Australia and New Zealand. British Museum (Natural History).

Nielsen, A. 1942. Uber die Entwicklung und Biologie der Trichopteren mit besonderer Berucksichtigung der Quelltrichopteren Himmerlands. Arch. Hydrobiol. Suppl. - Bd.17.

----- 1948. Post embryonic development and biology of the Hydrophilidac. Det. Kongelige dauske vidensk. Selskab, Biol. Skrifter, Vol.1.

----- 1957. A comparative study of the genital segments and their appendages in male trichoptera. Biol. Skr. Dan. Vid. Selsk. 8, no.5.

Orcutt, A.W. 1934. The Larvae of Trichoptera. In The Caddis Flies or Trichoptera of N.Y. State by Betten, 1934; see above.

Packard, A.S. 1909. Textbook of Entomology. New York.

Pantin, C.F.A. 1948. Notes on Microscopical Technique for Zoologists.

Powell, A.W.B. 1957. Shells of New Zealand.

Salmon, J.T. 1951. Polyvinyl alcohol as a mounting medium in microscopy. The Microscope, 8: 139 - 142.

----- 1955. Notes on Staining Techniques for Polyvinyl Alcohol Mountants. The Microscope, 10 (6).

Scherren, H. 1897. Rotifers commensal with caddis worms. Nature 56: 224.

Scott, W.N. 1936. An experimental analysis of the factors governing the hour of emergence of adult insects from their pupae. Trans. Roy. Ent. Soc. Lond., 85: 303 - 329.

Siltala, A.J. 1906. Uber den Laich der Trichopteren. Acta Soc. Fauna et Flora Fennica, 28: 4.

Slack, H.D. 1936. The food of the caddis fly. J. Anim. Ecol., 5: 105-115.

Snodgrass, R.E. 1935. Principles of Insect Morphology. McGraw Hill Book Company, New York.

Suter, H. 1913. Manual of the New Zealand Mollusca.

Tillyard, R.J. 1924. Studies of New Zealand Trichoptera. Trans. of New Zealand Institute, vol.55, p.285.

——— 1926. Insects of Australia and New Zealand.

Tindall, A.R. 1960. The larval case of Triaenodes bicolor Curtis. (Trichoptera: Leptoceridae). Proc. Roy. Ent. Soc. Lond., 35.

Tuxen, S.L. Ed. 1956. Taxonomists Glossary of Genitalia in Insects.

Ulmer, G. 1909. In part 5 and 6 of Die Süsswasserfauna Deutschlands, by A. Brauer.

Weaver, N. and Thomas, R.C. A fixative for use in dissecting insects. Stain Technology, vol.31, no.1. 1956

Wigglesworth, V.B. 1953. The Principles of Insect Physiology.

Wolfe, L.S. 1949. Studies on the genus Uropetala Selys (Odonata, Petaluridae) from New Zealand, with special reference to its life history, ecology, and systematics, and including studies on anatomical, histological and biochemical aspects. Unpublished Thesis, University of Canterbury.

## APPENDIX 1.

Monthly sample for August 1961. No. = 128.

Measurements in mm. H = hollow twig A = assorted plant material

| Head width | Case length | Larval length | Case composition | Head width | Case length | Larval length | Case composition |
|---|---|---|---|---|---|---|---|
| 1.3 | 25 | 12 | H | 1.4 | 38 | 18 | H |
| 1.2 | 30 | 13 | A | 1.4 | 24 | 14 | H |
| 0.5 | 14 | 6 | A | 0.8 | 10 | 7 | A |
| 0.6 | 11 | 6 | A | 1.4 | 30 | 18 | H |
| 1.2 | 23 | 16 | H | 1.2 | 23 | 15 | H |
| 1.0 | 18 | 11 | A | 1.4 | 27 | 18 | H |
| 1.4 | 20 | 16 | H | 1.2 | 26 | 16 | H |
| 1.2 | 16 | 9 | H | 1.5 | 29 | 20 | H |
| 0.5 | 12 | 6 | A | 0.8 | 16 | 10 | A |
| 0.8 | 9 | 6 | A | 1.4 | 30 | 16 | H |
| 1.2 | 18 | 9 | H | 1.4 | 25 | 17 | H |
| 1.2 | 17 | 10 | A | 1.4 | 25 | 18 | H |
| 1.4 | 23 | 17 | H | 1.4 | 29 | 18 | H |
| 1.4 | 25 | 14 | H | 1.2 | 23 | 15 | H |
| 0.9 | 28 | 8 | A | 1.4 | 28 | 19 | H |
| 0.8 | 22 | 10 | H | 1.2 | 25 | 12 | H |
| 0.9 | 22 | 10 | A | 1.4 | 40 | 12 | H |
| 1.2 | 18 | 14 | A | 1.2 | 29 | 17 | H |
| 1.4 | 18 | 12 | H | 1.4 | 26 | 19 | H |
| 0.8 | 20 | 8 | A | 1.2 | 15 | 19 | H |
| 1.2 | 20 | 15 | A | 1.4 | 26 | 17 | H |
| 0.9 | 28 | 12 | H | 1.5 | 27 | 19 | H |
| 1.2 | 17 | 16 | H | 1.2 | 17 | 9 | A |
| 1.2 | 28 | 12 | H | 1.2 | 10 | 22 | A |
| 1.0 | - | 10 | A | 1.2 | 23 | 15 | H |
| 1.3 | 30 | 15 | H | 1.4 | 38 | 20 | H |
| 1.4 | 38 | 18 | H | 1.2 | 20 | 15 | H |
| 1.2 | 28 | 15 | H | 1.4 | 29 | 14 | H |
| 0.9 | 15 | 10 | A | 1.2 | 16 | 14 | H |
| 0.8 | 25 | 11 | A | 0.8 | 11 | 9 | H |
| 0.8 | 15 | 8 | A | 1.4 | 15 | 11 | H |
| 1.4 | 38 | 16 | H | 1.3 | 17 | 11 | H |
| 0.8 | 34 | 10 | H | 1.2 | 22 | 15 | H |
| 1.4 | 30 | 12 | H | 1.2 | 19 | 15 | H |
| 1.4 | 30 | 14 | H | 1.2 | 30 | 16 | H |
| 1.4 | 28 | 18 | H | 1.3 | 35 | 18 | H |
| 1.2 | 24 | 15 | H | 1.4 | 25 | 18 | H |
| 1.4 | 30 | 19 | H | 1.4 | 20 | 17 | H |

| Head width | Case length | Larval length | Case composition | Head width | Case length | Larval length | Case composition |
|---|---|---|---|---|---|---|---|
| 1.4 | 28 | 20 | H | 1.4 | 25 | 17 | H |
| 1.4 | 38 | 17 | H | 1.4 | 35 | 17 | H |
| 1.4 | 38 | 19 | H | 1.2 | 22 | 14 | H |
| 1.2 | 18 | 14 | H | 1.2 | 25 | 16 | H |
| 1.4 | 18 | 16 | H | 1.3 | 27 | 19 | H |
| 1.2 | 20 | 14 | A | 1.4 | 35 | 18 | H |
| 1.2 | 20 | 13 | A | 1.4 | 38 | 16 | H |
| 1.2 | 26 | 16 | H | P | - | - | H |
| 1.2 | 21 | 15 | H | 0.9 | 16 | 8 | A |
| 1.2 | 30 | 15 | H | 1.2 | 25 | 15 | A |
| 1.4 | 32 | 16 | H | 1.2 | 15 | 16 | H |
| 1.4 | 30 | 19 | H | PP | - | - | H |
| 1.4 | 25 | 15 | H | 1.2 | 25 | 16 | H |
| 1.2 | 26 | 15 | H | 1.2 | 30 | 15 | H |
| 1.2 | 22 | 15 | H | 1.2 | 25 | 16 | A |
| 1.4 | 26 | 17 | H | 1.4 | 25 | 18 | H |
| 1.4 | 36 | 16 | H | 1.4 | 40 | 18 | H |
| 1.4 | 37 | 18 | H | 1.4 | 28 | 18 | A |
| 1.4 | 25 | 17 | H | 1.2 | 25 | 14 | H |
| 1.4 | 25 | 16 | H | 1.4 | 30 | 17 | H |
| 1.2 | 20 | 15 | H | 0.9 | 10 | 7 | A |
| 1.4 | 30 | 14 | H | 1.4 | 25 | 17 | A |
| 1.4 | 27 | 16 | H | 1.4 | 26 | 18 | H |
| 1.4 | 25 | 17 | H | 1.2 | 48 | 17 | H |
| 1.4 | 35 | 18 | H | 1.2 | 15 | 12 | H |
| 1.4 | 30 | 20 | H | 0.5 | 8 | 5 | H |